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E. BORNETT
Certifying Officer

A significant difference between the levels of endometrial marker levels in a patient and the normal levels is an indication that the patient is afflicted with or has a predisposition to endometrial disease.

In an embodiment the amount of endometrial marker(s) detected is greater than that of a standard and is indicative of endometrial disease, in particular endometrial cancer. In another embodiment the amount of endometrial marker(s) detected is lower than that of a standard and is indicative of endometrial disease, in particular endometrial cancer.

In another aspect, the invention provides a method for assessing the aggressiveness or indolence of an endometrial disease in particular cancer (e.g. staging), the method comprising comparing:

(a) levels of endometrial markers or polynucleotides encoding endometrial markers associated with the endometrial disease in a patient sample; and

(b) normal levels of the endometrial markers or the polynucleotides in a control sample.

A significant difference between the levels in the sample and the normal levels is an indication that the endometrial disease, in particular cancer, is aggressive or indolent. In an embodiment, the levels of endometrial markers are higher than normal levels. In another embodiment, the levels of endometrial markers are lower than normal levels.

In a particular embodiment, the invention provides a method for determining whether a cancer has metastasized or is likely to metastasize in the future, the method comprising comparing:

(a) levels of endometrial cancer markers or polynucleotides encoding endometrial cancer markers in a patient sample; and

(b) normal levels (or non-metastatic levels) of the endometrial cancer markers or polynucleotides in a control sample.

A significant difference between the levels in the patient sample and the normal levels is an indication that the cancer has metastasized or is likely to metastasize in the future.

In an aspect, the invention provides a method for monitoring the progression of endometrial disease, in particular endometrial cancer in a patient the method comprising:

(a) detecting endometrial markers or polynucleotides encoding the markers

associated with the disease in a sample from the patient at a first time point;

(b) repeating step (a) at a subsequent point in time; and

(c) comparing the levels detected in (a) and (b), and therefrom monitoring the progression of the endometrial disease.

The invention also provides a method for assessing the potential efficacy of a test agent for inhibiting endometrial disease, and a method of selecting an agent for inhibiting endometrial disease.

The invention also contemplates a method of assessing the potential of a test compound to contribute to an endometrial disease comprising:

(a) maintaining separate aliquots of endometrial diseased cells in the presence and absence of the test compound; and

(b) comparing the levels of endometrial markers or polynucleotides encoding the markers associated with the disease in each of the aliquots.

A significant difference between the levels of endometrial markers or polynucleotides encoding the markers in an aliquot maintained in the presence of (or exposed to) the test compound relative to the aliquot maintained in the absence of the test compound, indicates that the test compound potentially contributes to endometrial disease.

The invention further relates to a method of assessing the efficacy of a therapy for inhibiting endometrial disease in a patient. A method of the invention comprises comparing: (a) levels of endometrial markers or polynucleotides encoding the markers associated with disease in a sample from the patient obtained from the patient prior to providing at least a portion of the therapy to the patient; and (b) levels of endometrial markers or polynucleotides encoding the markers associated with disease in a second sample obtained from the patient following therapy.

A significant difference between the levels of endometrial markers or polynucleotides encoding the markers in the second sample relative to the first sample is an indication that the therapy is efficacious for inhibiting endometrial disease.

In an embodiment, the method is used to assess the efficacy of a therapy for inhibiting endometrial disease, where lower levels of endometrial markers or polynucleotides encoding same in the second sample relative to the first sample, is an

indication that the therapy is efficacious for inhibiting the disease.

The "therapy" may be any therapy for treating endometrial disease, in particular endometrial cancer, including but not limited to therapeutics, radiation, immunotherapy, gene therapy, and surgical removal of tissue. Therefore, the method can be used to evaluate a patient before, during, and after therapy.

Certain methods of the invention employ binding agents (e.g. antibodies) that specifically recognize endometrial markers.

In an embodiment, the invention provides methods for determining thè presence or absence of endometrial disease, in particular endometrial cancer, in a patient, comprising the steps of (a) contacting a biological sample obtained from a patient with one or more binding agent that specifically binds to one or more endometrial markers associated with the disease; and (b) detecting in the sample an amount of marker that binds to the binding agent, relative to a predetermined standard or cut-off value, and therefrom determining the presence or absence of endometrial disease in the patient.

In another embodiment, the invention relates to a method for diagnosing and monitoring an endometrial disease, in particular endometrial cancer, in a subject by quantitating one or more endometrial markers associated with the disease in a biological sample from the subject comprising (a) reacting the biological sample with one or more binding agent specific for the endometrial markers (e.g. an antibody) that are directly or indirectly labelled with a detectable substance; and (b) detecting the detectable substance.

In another aspect the invention provides a method for using an antibody to detect expression of one or more endometrial marker in a sample, the method comprising: (a) combining antibodies specific for one or more endometrial marker with a sample under conditions which allow the formation of antibody:marker complexes; and (b) detecting complex formation, wherein complex formation indicates expression of the marker in the sample. Expression may be compared with standards and is diagnostic of an endometrial disease, in particular endometrial cancer.

Embodiments of the methods of the invention involve (a) reacting a biological sample from a subject with antibodies specific for one or more endometrial markers which are directly or indirectly labelled with an enzyme; (b) adding a substrate for the enzyme wherein the substrate is selected so that the substrate, or a reaction product of the

enzyme and substrate forms fluorescent complexes; (c) quantitating one or more endometrial markers in the sample by measuring fluorescence of the fluorescent complexes; and (d) comparing the quantitated levels to levels obtained for other samples from the subject patient, or control subjects.

In another embodiment the quantitated levels are compared to levels quantitated for control subjects (e.g. normal or benign) without an endometrial disease (e.g. cancer) wherein an increase in endometrial marker levels compared with the control subjects is indicative of endometrial disease.

In further embodiment the quantitated levels are compared to levels quantitated for control subjects (e.g. normal or benign) without an endometrial disease (e.g. cancer) wherein a decrease in endometrial marker levels compared with the control subjects is indicative of endometrial disease.

A particular embodiment of the invention comprises the following steps

(a) incubating a biological sample with first antibodies specific for one or more endometrial cancer markers which are directly or indirectly labeled with a detectable substance, and second antibodies specific for one or more endometrial cancer markers which are immobilized;

(b) detecting the detectable substance thereby quantitating endometrial cancer markers in the biological sample; and

(c) comparing the quantitated endometrial cancer markers with levels for a predetermined standard.

The standard may correspond to levels quantitated for samples from control subjects without endometrial cancer (normal or benign), with a different disease stage, or from other samples of the subject. In an embodiment, increased levels of endometrial cancer markers as compared to the standard may be indicative of endometrial cancer. In another embodiment, lower levels of endometrial cancer markers as compared to the standard may be indicative of endometrial cancer.

Other methods of the invention employ one or more polynucleotides capable of hybridizing to one or more polynucleotides encoding endometrial markers. Thus, methods for detecting endometrial markers can be used to monitor an endometrial disease (e.g. cancer) by detecting endometrial polynucleotide markers associated with the disease.

Thus, the present invention relates to a method for diagnosing and monitoring an endometrial disease (e.g. endometrial cancer) in a sample from a subject comprising isolating nucleic acids, preferably mRNA, from the sample; and detecting endometrial marker polynucleotides associated with the disease in the sample. The presence of different levels of endometrial marker polynucleotides in the sample compared to a standard or control may be indicative of disease, endometrium phase, disease stage, and/or a positive prognosis i.e. longer progression-free and overall survival.

In an embodiment of the invention, endometrial cancer marker polynucleotide positive tumors (e.g. higher levels of the polynucleotides compared to a control normal or benign tissue) are a negative diagnostic indicator. Positive tumors can be indicative of endometrial cancer, advanced stage disease, lower progression-free survival, and/or overall survival.

In another embodiment of the invention, endometrial cancer marker polynucleotide negative tumors (e.g. lower levels of the polynucleotides compared to a control normal or benign tissue) are a negative diagnostic indicator. Negative tumors can be indicative of endometrial cancer, advanced stage disease, lower progression-free survival, and/or overall survival.

The invention provides methods for determining the presence or absence of an endometrial disease in a subject comprising detecting in the sample levels of nucleic acids that hybridize to one or more polynucleotides encoding endometrial markers associated with the disease, comparing the levels with a predetermined standard or cut-off value, and therefrom determining the presence or absence of endometrial disease in the subject. In an embodiment, the invention provides methods for determining the presence or absence of endometrial cancer in a subject comprising (a) contacting a sample obtained from the subject with oligonucleotides that hybridize to one or more polynucleotides encoding endometrial cancer markers; and (b) detecting in the sample a level of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, and therefrom determining the presence or absence of endometrial cancer in the subject.

Within certain embodiments, the amount of polynucleotides that are mRNA are detected via polymerase chain reaction using, for example, oligonucleotide primers that hybridize to one or more polynucleotides that encode endometrial markers, or

complements of such polynucleotides. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing oligonucleotide probes that hybridize to one or more polynucleotides that encode endometrial markers, or complements thereof.

When using mRNA detection, the method may be carried out by combining isolated mRNA with reagents to convert to cDNA according to standard methods; treating the converted cDNA with amplification reaction reagents (such as cDNA PCR reaction reagents) in a container along with an appropriate mixture of nucleic acid primers; reacting the contents of the container to produce amplification products; and analyzing the amplification products to detect the presence of one or more endometrial polynucleotide markers in the sample. For mRNA the analyzing step may be accomplished using Northern Blot analysis to detect the presence of endometrial polynucleotide markers. The analysis step may be further accomplished by quantitatively detecting the presence of endometrial polynucleotide markers in the amplification product, and comparing the quantity of marker detected against a panel of expected values for the known presence or absence of the markers in normal and malignant tissue derived using similar primers.

Therefore, the invention provides a method wherein mRNA is detected by (a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction reagents and nucleic acid primers that hybridize to one or more endometrial polynucleotide markers to produce amplification products; (d) analyzing the amplification products to detect an amount of mRNA encoding the endometrial markers; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and diseased tissue (e.g. malignant tissue) derived using similar nucleic acid primers.

The invention also contemplates a method comprising administering to cells or tissues imaging agents that carry labels for imaging and bind to endometrial markers and optionally other markers of an endometrial disease, and then imaging the cells or tissues.

In an aspect the invention provides an *in vivo* method comprising administering to a subject an agent that has been constructed to target one or more endometrial markers.

In a particular embodiment, the invention contemplates an *in vivo* method comprising administering to a mammal one or more agent that carries a label for imaging and binds to one or more endometrial marker, and then imaging the mammal.

According to a particular aspect of the invention, an *in vivo* method for imaging endometrial cancer is provided comprising:

(a) injecting a patient with an agent that binds to one or more endometrial cancer marker, the agent carrying a label for imaging the endometrial cancer;

(b) allowing the agent to incubate *in vivo* and bind to one or more endometrial cancer marker associated with the endometrial cancer; and

(c) detecting the presence of the label localized to the endometrial cancer.

In an embodiment of the invention the agent is an antibody which recognizes an endometrial cancer marker. In another embodiment of the invention the agent is a chemical entity which recognizes an endometrial cancer marker.

An agent carries a label to image an endometrial marker and optionally other markers. Examples of labels useful for imaging are radiolabels, fluorescent labels (e.g fluorescein and rhodamine), nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic markers such as peroxidase or phosphatase. Short-range radiation emitters, such as isotopes detectable by short-range detector probes can also be employed.

The invention also contemplates the localization or imaging methods described herein using multiple markers for an endometrial disease (e.g. endometrial cancer).

The invention also relates to kits for carrying out the methods of the invention. In an embodiment, the kit is for assessing whether a patient is afflicted with an endometrial disease (e.g. endometrial cancer) and it comprises reagents for assessing one or more endometrial markers or polynucleotides encoding the markers.

The invention also provides a diagnostic composition comprising an endometrial marker or a polynucleotide encoding the marker. A composition is also provided comprising a probe that specifically hybridizes to endometrial polynucleotide markers, or a fragment thereof, or an antibody specific for endometrial markers or a fragment thereof. In another aspect, a composition is provided comprising one or more

endometrial polynucleotide marker specific primer pairs capable of amplifying the polynucleotides using polymerase chain reaction methodologies. The probes, primers or antibodies can be labeled with a detectable substance.

Still further the invention relates to therapeutic applications for endometrial diseases, in particular endometrial cancer, employing endometrial markers and polynucleotides encoding the markers, and/or binding agents for the markers.

In an aspect, the invention relates to compositions comprising endometrial markers or parts thereof associated with an endometrial disease, or antibodies specific for endometrial markers associated with an endometrial disease, and a pharmaceutically acceptable carrier, excipient, or diluent. A method for treating or preventing an endometrial disease, in particular endometrial cancer, in a patient is also provided comprising administering to a patient in need thereof, endometrial markers or parts thereof associated with an endometrial disease, antibodies specific for endometrial markers associated with an endometrial disease, or a composition of the invention. In an aspect the invention provides a method of treating a patient afflicted with or at risk of developing an endometrial disease (e.g. endometrial cancer) comprising inhibiting expression of endometrial markers.

In an aspect, the invention provides antibodies specific for endometrial markers associated with a disease (e.g. endometrial cancer) that can be used therapeutically to destroy or inhibit the disease (e.g. the growth of endometrial cancer marker expressing cancer cells), or to block endometrial marker activity associated with a disease. In an aspect, endometrial cancer markers may be used in various immunotherapeutic methods to promote immune-mediated destruction or growth inhibition of tumors expressing endometrial cancer markers.

The invention also contemplates a method of using endometrial markers or parts thereof, or antibodies specific for endometrial markers in the preparation or manufacture of a medicament for the prevention or treatment of an endometrial disease e.g. endometrial cancer.

Another aspect of the invention is the use of endometrial markers, peptides derived therefrom, or chemically produced (synthetic) peptides, or any combination of these molecules, for use in the preparation of vaccines to prevent an endometrial disease and/or to treat an endometrial disease.

The invention contemplates vaccines for stimulating or enhancing in a subject to whom the vaccine is administered production of antibodies directed against one or more endometrial markers.

The invention also provides a method for stimulating or enhancing in a subject production of antibodies directed against one or more endometrial marker. The method comprises administering to the subject a vaccine of the invention in a dose effective for stimulating or enhancing production of the antibodies.

The invention further provides a method for treating, preventing, or delaying recurrence of an endometrial disease (e.g. endometrial cancer). The method comprises administering to the subject a vaccine of the invention in a dose effective for treating, preventing, or delaying recurrence of an endometrial disease (e.g. endometrial cancer).

The invention contemplates the methods, compositions, and kits described herein using additional markers associated with an endometrial disease (e.g. endometrial cancer). The methods described herein may be modified by including reagents to detect the additional markers, or polynucleotides for the markers.

In particular, the invention contemplates the methods described herein using multiple markers for an endometrial cancer. Therefore, the invention contemplates a method for anaylzing a biological sample for the presence of endometrial cancer markers and polynucleotides encoding the markers, and other markers that are specific indicators of cancer, in particular endometrial cancer. The methods described herein may be modified by including reagents to detect the additional markers, or nucleic acids for the additional markers.

In embodiments of the invention the methods, compositions and kits use one or more of the markers listed in Table 1. In another embodiment, the method uses a panel of markers selected from the markers listed in Table 1, in particular a panel comprising two or more of the markers in Table 1.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 shows a schematic representation of the ICAT procedure including labeling, digestion, SCX fractionation, affinity isolation, cleavage of the biotin moiety and LC-MS/MS analysis.

Figure 2 shows histologic sections of human endometrium, stained with hematoxylin and eosin. A) proliferative endometrium, sample PRO 2, and B) secretory endometrium, sample SEC2.

Figure 3 shows a NanoLC-MS TIC of SCX fraction 16, ICAT sample A.

Figure 4 shows the distribution of proteins identified from the middle ten SCX fractions of all three samples.

Figure 5 shows A) a mass spectral window of Sample A, SCX fraction 19, showing enhanced expression of glutamate receptor subunit zeta 1 precursor in the secretory endometrium. The line to the left of the isotope envelope of the heavy-labeled version of the protein denotes where the light-labeled version is expected. B) Resulting MS/MS spectra of the heavy labelled series showing sequence coverage.

Figure 6 shows A) a mass spectral window of Sample A, SCX fraction 13, showing enhanced expression of MIF in the secretory endometrium. B) Resulting MS/MS spectra of the heavy labelled series sequence coverage.

Figure 7 (a+b). STEP ONE Mass spectra obtained from five whole endometrial tissue homogeneates on Proteinchip WCX2 using (1a) SELDI-TOF-MS, PBSIIc, and (1b) QqTOF-MS, QSTAR XL. Note, using either MS method, the three malignant cases (38, 39, 40) show a distinct protein peak, which is low in the two non-malignant cases (18, 19). The QSTARXL MS determined the weight of this peak to be 10,843 Da. Figure 7 STEP TWO Outline of protein purification. The target protein eluted in one of the early fractions of the size exclusion HPLC, was concentrated by Proteospin and then subjected to MALDI and SDS-PAGE (see Figure 8).

Figure 8 (A to E). STEP THREE (8A). Molecular weight verification of target protein after SEC. MALDI-TOF MS of samples from Proteospin was used to locate the fraction with the 10,843 Da weight of the target protein in a malignant case (40).The corresponding fraction from normal sample was used in parallel for all the following experiments. (8B) Molecular weight verification of the target protein fraction following

DTT treatment. MALDI-TOF analysis of two samples indicates that the 10,843 Da peak remained in the malignant sample even after DTT treatment. (8C) SDS-PAGE analysis of the DTT treated fractions. The gel was stained by colloidal coomassie blue (Gel-Code, Pierce). The region containing the target protein was excised according to the molecular weight marker, and the intact proteins were extracted. (8D) Tryptic peptide profiles of the SDS-PAGE protein extract. MALDI-TOF MS analysis identified six "unique" tryptic peptides in the malignant samples. Three of them with mass of 907.54, 1036.58, and 1529.82 Da matched with the tryptic peptides from chaperonin 10. The remaining three peaks, marked with *, were from Keratin. (insert) Amino acid sequence of chaperonin 10. The locations of the three peptides were underlined. (8E) Fragmentation of peptide by collision induced dissociation. The 1529.82 Da peptide were fragmented. A series of y ions confirmed that the 1529.82 Da peptide comes from chaperonin 10.

Figure 9. Western blot analysis against chaperonin 10. The malignant endometrial cases (EmCa) demonstrate a higher expression of chaperonin 10 than non-malignant cases (Normal). The level of ERK1 was used to demonstrate similar loading among all lanes.

Figure 10. Immunohistochemical staining for chaperonin 10. Note granular cytoplasmic positivity in several malignant endometrioid glands, while adjacent endometrial stroma and a portion of one benign endometrial gland (right) shows much reduced staining.

Figure 11 shows mass spectral windows from isotope-coded affinity tag (ICAT) experiments for three pairs of endometrial cancer / normal samples, demonstrating the over expression of calgizzarin in the cancer samples.

DETAILED DESCRIPTION OF THE INVENTION

Methods are provided for characterizing the stage or phase of endometrium, detecting the presence of an endometrial disease (e.g. endometrial cancer) in a sample, the absence of a disease (e.g. endometrial cancer) in a sample, the stage of a disease, the stage or grade of the disease, and other characteristics of endometrial diseases that are relevant to prevention, diagnosis, characterization, and therapy of cancer in a patient, for example, the benign or malignant nature of an endometrial cancer, the metastatic potential of an endometrial cancer, assessing the histological type of neoplasm associated with an endometrial cancer, the indolence or aggressiveness of an endometrial cancer,

and other characteristics of endometrial diseases that are relevant to prevention, diagnosis, characterization, and therapy of cancer in a patient. Methods are also provided for assessing the efficacy of one or more test agents for inhibiting an endometrial disease, assessing the efficacy of a therapy for an endometrial disease, monitoring the progression of an endometrial disease, selecting an agent or therapy for inhibiting an endometrial disease, treating a patient afflicted with an endometrial disease, inhibiting an endometrial disease in a patient, and assessing the disease (e.g. carcinogenic) potential of a test compound.

Glossary

"Endometrial disease" refers to any disorder, disease, condition, syndrome or combination of manifestations or symptoms recognized or diagnosed as a disorder of the endometrium, including but not limited to hyperplasia and cancer precursors, endometrial cancer or carcinoma, endometriosis, reproductive disorders, and infertility.

"Endometrial cancer" or "endometrial carcinoma" includes malignant endometrial disease including but not limited to endometrioid, mucinous, and serous adenocarcinomas, adenosquamous carcinomas, clear cell carcinomas, uterine sarcomas including stromal sarcomas, malignant mixed Mullerian tumors (carcinosarcomas), and leiomyosarcomas.

The terms "sample", "biological sample", and the like mean a material known or suspected of expressing or containing endometrial polynucleotide markers or one or more endometrial markers. A test sample can be used directly as obtained from the source or following a pretreatment to modify the character of the sample. The sample can be derived from any biological source, such as tissues, extracts, or cell cultures, including cells (e.g. tumor cells), cell lysates, and physiological fluids, such as, for example, whole blood, plasma, serum, saliva, ocular lens fluid, cerebral spinal fluid, sweat, urine, milk, ascites fluid, synovial fluid, peritoneal fluid, lavage fluid, and the like. The sample can be obtained from animals, preferably mammals, most preferably humans. The sample can be treated prior to use, such as preparing plasma from blood, diluting viscous fluids, and the like. Methods of treatment can involve filtration, distillation, extraction, concentration, inactivation of interfering components, the addition of reagents, and the like.

In embodiments of the invention the sample is a mammalian tissue sample. In a

particular embodiment, the tissue is endometrial tissue.

In another embodiment the sample is a human physiological fluid. In a particular embodiment, the sample is human serum.

The terms "subject", "individual" or "patient" refer to a warm-blooded animal such as a mammal. In particular, the terms refer to a human. A subject, individual or patient may be afflicted with or suspected of having or being pre-disposed to endometrial disease or a condition as described herein.

The term "endometrial marker" includes a marker associated with normal or diseased endometrial tissue identified using a method of the invention. The term includes native-sequence polypeptides isoforms, chimeric polypeptides, complexes, all homologs, fragments, precursors, and modified forms and derivatives of the markers. The marker may be associated with a stage or phase of endometrial tissue such as the secretory or proliferative phase. Examples of endometrial markers associated with the secretory phase are glutamate receptor subunit zeta 1 [SEQ ID NO. 21] and parts thereof (e.g. a tryptic fragment of SEQ ID NO. 23], and macrophage migration inhibitory factor [SEQ ID NO. 15].

In an aspect of the invention the endometrial marker is associated with an endometrial disease, in particular it may be an endometrial cancer marker. The term "endometrial cancer marker" includes a marker associated with endometrial cancer identified using a method of the invention, in particular a marker listed in Table 1.

In an aspect of the invention, the endometrial cancer marker is chaperonin 10. The term "chaperonin 10", "chaperonin 10 polypeptide" or "chaperonin protein" includes human chaperonin 10, in particular the native-sequence polypeptide, isoforms, chimeric polypeptides, all homologs, fragments, precursors, complexes, and modified forms and derivatives of human chaperonin 10. The amino acid sequence for native human chaperonin 10 includes the sequences of GenBank Accession No. QO4984 and shown in SEQ ID NO. 1.

A "native-sequence polypeptide" comprises a polypeptide having the same amino acid sequence of a polypeptide derived from nature. Such native-sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term specifically encompasses naturally occurring truncated or secreted forms of a

polypeptide, polypeptide variants including naturally occurring variant forms (e.g. alternatively spliced forms or splice variants), and naturally occurring allelic variants.

The term "polypeptide variant" means a polypeptide having at least about 70-80%, preferably at least about 85%, more preferably at least about 90%, most preferably at least about 95% amino acid sequence identity with a native-sequence polypeptide. Particular polypeptide variants have at least 70-80%, 85%, 90%, 95% amino acid sequence identity to the sequences identified in Table 1 (GenBank Accession Nos. Q04984, P05109, P06702, P01833 or Q81ZY7, P30086, P39687, P17066, P14174, P31949, P00938 and SEQ ID NOs. 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19). Such variants include, for instance, polypeptides wherein one or more amino acid residues are added to, or deleted from, the N- or C-terminus of the full-length or mature sequences of the polypeptide, including variants from other species, but excludes a native-sequence polypeptide.

An allelic variant may also be created by introducing substitutions, additions, or deletions into a polynucleotide encoding a native polypeptide sequence such that one or more amino acid substitutions, additions, or deletions are introduced into the encoded protein. Mutations may be introduced by standard methods, such as site-directed mutagenesis and PCR-mediated mutagenesis. In an embodiment, conservative substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which an amino acid residue is replaced with an amino acid residue with a similar side chain. Amino acids with similar side chains are known in the art and include amino acids with basic side chains (e.g. Lys, Arg, His), acidic side chains (e.g. Asp, Glu), uncharged polar side chains (e.g. Gly, Asp, Glu, Ser, Thr, Tyr and Cys), nonpolar side chains (e.g. Ala, Val, Leu, Iso, Pro, Trp), beta-branched side chains (e.g. Thr, Val, Iso), and aromatic side chains (e.g. Tyr, Phe, Trp, His). Mutations can also be introduced randomly along part or all of the native sequence, for example, by saturation mutagenesis. Following mutagenesis the variant polypeptide can be recombinantly expressed and the activity of the polypeptide may be determined.

Polypeptide variants include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of a native polypeptide which include fewer amino acids than the full length polypeptides. A portion of a

polypeptide can be a polypeptide which is for example, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more amino acids in length. Portions in which regions of a polypeptide are deleted can be prepared by recombinant techniques and can be evaluated for one or more functional activities such as the ability to form antibodies specific for a polypeptide.

A naturally occurring allelic variant may contain conservative amino acid substitutions from the native polypeptide sequence or it may contain a substitution of an amino acid from a corresponding position in a polypeptide homolog, for example, a murine polypeptide.

The invention also includes polypeptides that are substantially identical to the sequences of an endometrial marker, in particular an endometrial cancer marker, more particularly a marker listed in Table 1 [GenBank Accession Nos. Q04984, P05109, P06702, P01833 or Q81ZY7, P30086, P39687, P17066, P14174, P31949, P00938 and SEQ ID NOs. 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19] (e.g. at least about 45%, preferably 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% sequence identity), and in particular polypeptides that retain the immunogenic activity of the corresponding native-sequence polypeptide.

Percent identity of two amino acid sequences, or of two nucleic acid sequences is defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues in a polypeptide or nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid or nucleic acid sequence identity can be achieved in various conventional ways, for instance, using publicly available computer software including the GCG program package (Devereux J. et al., Nucleic Acids Research 12(1): 387, 1984); BLASTP, BLASTN, and FASTA (Atschul, S.F. et al. J. Molec. Biol. 215: 403-410, 1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S. et al. NCBI NLM NIH Bethesda, Md. 20894; Altschul, S. et al. J. Mol. Biol. 215: 403-410, 1990). Skilled artisans can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Methods to determine identity and

similarity are codified in publicly available computer programs.

Endometrial markers include chimeric or fusion proteins. A "chimeric protein" or "fusion protein" comprises all or part (preferably biologically active) of an endometrial marker operably linked to a heterologous polypeptide (i.e., a polypeptide other than an endometrial marker). Within the fusion protein, the term "operably linked" is intended to indicate that an endometrial marker and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of an endometrial marker. A useful fusion protein is a GST fusion protein in which an endometrial marker is fused to the C-terminus of GST sequences. Another example of a fusion protein is an immunoglobulin fusion protein in which all or part of an endometrial marker is fused to sequences derived from a member of the immunoglobulin protein family. Chimeric and fusion proteins can be produced by standard recombinant DNA techniques.

A modified form of a polypeptide referenced herein includes modified forms of the polypeptides and derivatives of the polypeptides, including but not limited to glycosylated, phosphorylated, acetylated, methylated or lapidated forms of the polypeptides. For example, an N-terminal methionine may be cleaved from a polypeptide, and a new N-terminal residue may or may not be acetylated.

Endometrial markers identified in accordance with a method of the invention may be prepared by recombinant or synthetic methods, or isolated from a variety of sources, or by any combination of these and similar techniques.

"Endometrial polynucleotide marker(s)" refers to polynucleotides encoding endometrial markers including native-sequence polypeptides, polypeptide variants including a portion of a polypeptide, an isoform, precursor, complex, a chimeric polypeptide, or modified forms and derivatives of the polypeptides. A polynucleotide encoding a native polypeptide employed in the present invention includes the polynucleotides encoding glutamate receptor subunit zeta 1 [Accession No. D13515 and SEQ ID NO. 22], a tryptic peptide thereof [SEQ ID NO. 23], and macrophage migration inhibitory factor [Accession No. NM_002415 and SEQ ID NO. 16] and the markers listed in Table 1 [GenBank Accession Nos. NM_002157 and HSU07550, NM_002964 and BT007378, X06233, NM_002644, BC031102, X75090.1, NM_002155, NM_002415, NM_005620 and NM_00365, and SEQ ID NOs. 2, 4, 6, 8, 10, 12, 14, 16,

18, and 20]. In a particular embodiment, a polynucleotide of the invention encodes chaperonin 10, more particularly a polynucleotide sequence of GenBank Accession No. NM_002157 [SEQ ID NO 2], or a fragment thereof.

Endometrial polynucleotide markers include complementary nucleic acid sequences, and nucleic acids that are substantially identical to these sequences (e.g. at least about 45%, preferably 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% sequence identity).

Endometrial polynucleotide markers also include sequences that differ from a native sequence [e.g. GenBank Accession Nos. NM_002157 and HSU07550, NM_002964 and BT007378, X06233, NM_002644, BC031102, X75090.1, NM_002155, NM_002415, NM_005620 and NM_00365, and SEQ ID NOs. 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 and SEQ ID NOs. 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, respectively] due to degeneracy in the genetic code. As one example, DNA sequence polymorphisms within the nucleotide sequence of an endometrial marker may result in silent mutations that do not affect the amino acid sequence. Variations in one or more nucleotides may exist among individuals within a population due to natural allelic variation. DNA sequence polymorphisms may also occur which lead to changes in the amino acid sequence of a polypeptide.

Endometrial polynucleotide markers also include nucleic acids that hybridize under stringent conditions, preferably high stringency conditions to an endometrial cancer marker polynucleotide [e.g. GenBank Accession Nos. NM_002157 and HSU07550, NM_002964 and BT007378, X06233, NM_002644, BC031102, X75090.1, NM_002155, NM_002415, NM_005620 and NM_00365, and SEQ ID NOs. 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 and SEQ ID NOs. 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20, respectively]. Appropriate stringency conditions which promote DNA hybridization are known to those skilled in the art, or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. For example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2.0 x SSC at 50°C may be employed. The stringency may be selected based on the conditions used in the wash step. By way of example, the salt concentration in the wash step can be selected from a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be at high stringency conditions, at about 65°C.

Endometrial polynucleotide markers also include truncated nucleic acids or nucleic acid fragments and variant forms of the nucleic acids that arise by alternative splicing of an mRNA corresponding to a DNA.

The endometrial polynucleotide markers are intended to include DNA and RNA (e.g. mRNA) and can be either double stranded or single stranded. A polynucleotide may, but need not, include additional coding or non-coding sequences, or it may, but need not, be linked to other molecules and/or carrier or support materials. The polynucleotides for use in the methods of the invention may be of any length suitable for a particular method. In certain applications the term refers to antisense polynucleotides (e.g. mRNA or DNA strand in the reverse orientation to sense cancer polynucleotide markers).

"Statistically different levels", "significantly altered levels", or "significant difference" in levels of markers in a patient sample compared to a control or standard (e.g. normal levels or levels in other samples from a patient) may represent levels that are higher or lower than the standard error of the detection assay. In particular embodiments, the levels may be 1.5, 2, 3, 4, 5, or 6 times higher or lower than the control or standard.

"Binding agent" refers to a substance such as a polypeptide or antibody that specifically binds to one or more endometrial markers. A substance "specifically binds" to one or more endometrial markers if is reacts at a detectable level with one or more endometrial markers, and does not react detectably with peptides containing an unrelated or different sequence. Binding properties may be assessed using an ELISA, which may be readily performed by those skilled in the art (see for example, Newton et al , Develop. Dynamics 197: 1-13, 1993).

A binding agent may be a ribosome, with or without a peptide component, an aptamer, an RNA molecule, or a polypeptide. A binding agent may be a polypeptide that comprises one or more endometrial marker sequence, a peptide variant thereof, or a non-peptide mimetic of such a sequence. By way of example, a chaperonin 10 sequence may be a peptide portion of a chaperonin 10 that is capable of modulating a function mediated by chaperonin 10.

An aptamer includes a DNA or RNA molecule that binds to nucleic acids and proteins. An aptamer that binds to a protein (or binding domain) of an endometrial marker or an endometrial polynucleotide marker can be produced using conventional

techniques, without undue experimentation. [For example, see the following publications describing *in vitro* selection of aptamers: Klug et al., Mol. Biol. Reports 20:97-107 (1994); Wallis et al., Chem. Biol. 2:543-552 (1995); Ellington, Curr. Biol. 4:427-429 (1994); Lato et al., Chem. Biol. 2:291-303 (1995); Conrad et al., Mol. Div. 1:69-78 (1995); and Uphoff et al., Curr. Opin. Struct. Biol. 6:281-287 (1996)].

Antibodies for use in the present invention include but are not limited to monoclonal or polyclonal antibodies, immunologically active fragments (e.g. a Fab or $(Fab)_2$ fragments), antibody heavy chains, humanized antibodies, antibody light chains, genetically engineered single chain F_v molecules (Ladner et al, U.S. Pat. No. 4,946,778), chimeric antibodies, for example, antibodies which contain the binding specificity of murine antibodies, but in which the remaining portions are of human origin, or derivatives, such as enzyme conjugates or labeled derivatives.

Antibodies including monoclonal and polyclonal antibodies, fragments and chimeras, may be prepared using methods known to those skilled in the art. Isolated native or recombinant endometrial markers may be utilized to prepare antibodies. See, for example, Kohler et al. (1975) Nature 256:495-497; Kozbor et al. (1985) J. Immunol Methods 81:31-42; Cote et al. (1983) Proc Natl Acad Sci 80:2026-2030; and Cole et al. (1984) Mol Cell Biol 62:109-120 for the preparation of monoclonal antibodies; Huse et al. (1989) Science 246:1275-1281 for the preparation of monoclonal Fab fragments; and, Pound (1998) Immunochemical Protocols, Humana Press, Totowa, N.J for the preparation of phagemid or B-lymphocyte immunoglobulin libraries to identify antibodies. Antibodies specific for an endometrial marker may also be obtained from scientific or commercial sources.

In an embodiment of the invention, antibodies are reactive against an endometrial marker if they bind with a K_a of greater than or equal to 10^{-7} M.

Methods for Identifying Endometrial Markers

The invention relates to a method for identifying markers associated with the endometrium or a phase thereof, or associated with a disease of the endometrium comprising:

(a) obtaining a sample of endometrium from a subject;

(b) extracting proteins from the sample and producing a profile of the proteins by subjecting the proteins to mass spectrometry; and

(c) comparing the profile with a profile for normal endometrial tissue or for a known endometrium phase to identify proteins associated with the endometrium phase or an endometrial disease.

Proteins may be extracted from the samples in a manner known in the art. For example, proteins may be extracted by first digesting or disrupting cell membranes by standard methods such as detergents or homogenization in an isotonic sucrose solution, followed by ultra-centrifugation or other standard techniques.

The separated proteins may be digested into peptides, in particular using proteolytic enzymes such as trypsin, pepsin, subtilisin, and proteinase. For example, proteins may be treated with trypsin which cleaves at the sites of lysine and arginine, to provide doubly-charged peptides with a length of from about 5 to 50 amino acids. Such peptides may be particularly appropriate for mass spectrometry analysis, especially electrospray ionization mass spectrometry. Chemical reagents including cyanogens bromide may also be utilized to digest proteins.

Mass spectrometers that may be used to analyze the peptides or proteins include a Matrix-Assisted Laser Desorptioon/Ioniation Time-of-Flight Mass Spectrometer ("MALDI-TOF") (e.g. from PerSeptive Biosystems, Framingham, Mass.); an Electrospray Ionization ("ESI") ion trap spectrometer, (e.g. from Finnigan MAT, San Jose, Calif.), an ESI quadrupole mass spectrometer (e.g. from Finnigan or Perkin-Elmer Corporation, Foster City, Calif.), a quadrupole/TOF hybrid tandem mass spectrometer, QSTAR XL (Applied Biosystems/MDS Sciex), or a Surface Enhanced Laser Desorption/Ionization (SELDI-TOF) Mass Spectrometer (e.g. from Ciphergen Biosystems Inc.).

Detection Methods

A variety of methods can be employed for the diagnostic and prognostic evaluation of endometrial disease or endometrial status involving one or more endometrial markers and polynucleotides encoding the markers, and the identification of subjects with a predisposition to endometrial diseases or that are receptive to *in vitro* fertilization and embryo transfer procedures. Such methods may, for example, utilize endometrial polynucleotide markers, and fragments thereof, and binding agents (e.g. antibodies) against one or more endometrial markers, including peptide fragments. In particular, the polynucleotides and antibodies may be used, for example, for (1) the

detection of the presence of endometrial polynucleotide marker mutations, or the detection of either over- or under-expression of endometrial marker mRNA relative to a non-disorder state or different endometrium phase, or the qualitative or quantitative detection of alternatively spliced forms of endometrial polynucleotide marker transcripts which may correlate with certain conditions or susceptibility toward such conditions; and (2) the detection of either an over- or an under-abundance of one or more endometrial markers relative to a non- disorder state or a different endometrium phase or the presence of a modified (e.g., less than full length) endometrial marker which correlates with a disorder state or a progression toward a disorder state, or a particular endometrium phase.

The invention contemplates a method for detecting the phase of an endometrial tissue, in particular a secretory endometrial tissue, comprising producing a profile of levels of one or more endometrial marker associated with a known endometrium phase and/or polynucleotides encoding the markers, and optionally other markers associated with the endometrium phase in cells from a patient, and comparing the profile with a reference to identify a profile for the test cells indicative of the endometrium phase.

The invention also contemplates a method for detecting an endometrial disease, in particular an endometrial cancer, comprising producing a profile of levels of one or more endometrial marker associated with an endometrial disease and/or polynucleotides encoding the markers, and other markers associated with endometrial disease in cells from a patient, and comparing the profile with a reference to identify a profile for the test cells indicative of disease.

The methods described herein may be used to evaluate the probability of the presence of malignant or pre-malignant cells, for example, in a group of cells freshly removed from a host. Such methods can be used to detect tumors, quantitate their growth, and help in the diagnosis and prognosis of disease. The methods can be used to detect the presence of cancer metastasis, as well as confirm the absence or removal of all tumor tissue following surgery, cancer chemotherapy, and/or radiation therapy. They can further be used to monitor cancer chemotherapy and tumor reappearance.

The methods described herein can be adapted for diagnosing and monitoring endometrial tissue status or an endometrial disease by detecting one or more endometrial markers or polynucleotides encoding the markers in biological samples from a subject.

These applications require that the amount of markers or polynucleotides quantitated in a sample from a subject being tested be compared to a predetermined standard or cut-off value. The standard may correspond to levels quantitated for another sample or an earlier sample from the subject, or levels quantitated for a control sample. Levels for control samples from healthy subjects, different endometrial tissue phases, or subjects with an endometrial disease may be established by prospective and/or retrospective statistical studies. Healthy subjects who have no clinically evident disease or abnormalities may be selected for statistical studies. Diagnosis may be made by a finding of statistically different levels of detected endometrial markers associated with disease or polynucleotides encoding same, compared to a control sample or previous levels quantitated for the same subject.

The methods described herein may also use multiple markers for an endometrial disease, in particular endometrial cancer. Therefore, the invention contemplates a method for anaylzing a biological sample for the presence of one or more endometrial markers and polynucleotides encoding the markers, and other markers that are specific indicators of an endometrial disease. The methods described herein may be modified by including reagents to detect the additional markers, or polynucleotides for the markers.

Nucleic Acid Methods/Assays

As noted herein an endometrial disease or phase may be detected based on the level of endometrial polynucleotide markers in a sample. Techniques for detecting polynucleotides such as polymerase chain reaction (PCR) and hybridization assays are well known in the art.

Probes may be used in hybridization techniques to detect endometrial polynucleotide markers. The technique generally involves contacting and incubating nucleic acids (e.g. recombinant DNA molecules, cloned genes) obtained from a sample from a patient or other cellular source with a probe under conditions favorable for the specific annealing of the probes to complementary sequences in the nucleic acids. After incubation, the non-annealed nucleic acids are removed, and the presence of nucleic acids that have hybridized to the probe if any are detected.

Nucleotide probes for use in the detection of nucleic acid sequences in samples may be constructed using conventional methods known in the art. Suitable probes may be based on nucleic acid sequences encoding at least 5 sequential amino acids from

regions of an endometrial polynucleotide marker, preferably they comprise 15 to 40 nucleotides. A nucleotide probe may be labeled with a detectable substance such as a radioactive label that provides for an adequate signal and has sufficient half-life such as ^{32}P, ^{3}H, ^{14}C or the like. Other detectable substances that may be used include antigens that are recognized by a specific labeled antibody, fluorescent compounds, enzymes, antibodies specific for a labeled antigen, and luminescent compounds. An appropriate label may be selected having regard to the rate of hybridization and binding of the probe to the nucleotide to be detected and the amount of nucleotide available for hybridization. Labeled probes may be hybridized to nucleic acids on solid supports such as nitrocellulose filters or nylon membranes as generally described in Sambrook et al, 1989, Molecular Cloning, A Laboratory Manual (2nd ed.). The nucleic acid probes may be used to detect endometrial polynucleotide markers, preferably in human cells. The nucleotide probes may also be useful in the diagnosis of an endometrial disease involving one or more endometrial polynucleotide markers; in monitoring the progression of such disorder; or monitoring a therapeutic treatment.

The detection of endometrial polynucleotide markers may involve the amplification of specific gene sequences using an amplification method such as polymerase chain reaction (PCR), followed by the analysis of the amplified molecules using techniques known to those skilled in the art. Suitable primers can be routinely designed by one of skill in the art.

By way of example, at least two oligonucleotide primers may be employed in a PCR based assay to amplify a portion of a polynculeotide encoding one or more endometrial marker derived from a sample, wherein at least one of the oligonucleotide primers is specific for (i.e. hybridizes to) a polynucleotide encoding the endometrial marker. The amplified cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis.

In order to maximize hybridization under assay conditions, primers and probes employed in the methods of the invention generally have at least about 60%, preferably at least about 75%, and more preferably at least about 90% identity to a portion of a polynucleotide encoding an endometrial marker; that is, they are at least 10 nucleotides, and preferably at least 20 nucleotides in length. In an embodiment the primers and probes are at least about 10-40 nucleotides in length.

Hybridization and amplification techniques described herein may be used to assay qualitative and quantitative aspects of endometrial polynucleotide marker expression. For example, RNA may be isolated from a cell type or tissue known to express an endometrial polynucleotide marker and tested utilizing the hybridization (e.g. standard Northern analyses) or PCR techniques referred to herein.

The primers and probes may be used in the above-described methods *in situ* i.e directly on tissue sections (fixed and/or frozen) of patient tissue obtained from biopsies or resections.

In an aspect of the invention, a method is provided employing reverse transcriptase-polymerase chain reaction (RT-PCR), in which PCR is applied in combination with reverse transcription. Generally, RNA is extracted from a sample tissue using standard techinques (for example, guanidine isothiocyanate extraction as described by Chomcynski and Sacchi, Anal. Biochem. 162:156-159, 1987) and is reverse transcribed to produce cDNA. The cDNA is used as a template for a polymerase chain reaction. The cDNA is hybridized to a set of primers, at least one of which is specifically designed against an endometrial marker sequence. Once the pimer and template have annealed a DNA polymerase is employed to extend from the primer, to synthesize a copy of the template. The DNA strands are denatured, and the procedure is repeated many times until sufficient DNA is generated to allow visualization by ethidium bromide staining and agarose gel electrophoresis.

Amplification may be performed on samples obtained from a subject with a suspected endometrial disease and an individual who is not afflicted with an endometrial disease. The reaction may be performed on several dilutions of cDNA spanning at least two orders of magnitude. A statistically significant difference in expression in several dilutions of the subject sample as compared to the same dilutions of the non-disease sample may be considered positive for the presence of an endometrial disease.

In an embodiment, the invention provides methods for determining the presence or absence of an endometrial disease in a subject comprising (a) contacting a sample obtained from the subject with oligonucleotides that hybridize to endometrial marker polynucleotides; and (b) detecting in the sample a level of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, and therefrom determining the presence or absence of an endometrial disease in the subject.

In another embodiment, the invention provides methods for determining uterine receptivity of a subject to *in vitro* fertilization comprising (a) contacting a sample obtained from the subject with oligonucleotides that hybridize to endometrial marker polynucleotides associated with an endometrial tissue phase (e.g. secretory phase); and (b) detecting in the sample a level of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, wherein the presence or absence of the endometrial marker polynucleotides as compared to non-receptive controls indicates uterine receptivity.

The invention provides a method wherein an endometrial marker mRNA is detected by (a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction reagents and nucleic acid primers that hybridize to one or more endometrial marker polynucleotides, to produce amplification products; (d) analyzing the amplification products to detect amounts of mRNA encoding endometrial polynucleotide markers; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and malignant tissue derived using similar nucleic acid primers.

Endometrial cancer marker-positive samples or alternatively higher levels in patients compared to a control (e.g. non-cancerous tissue) may be indicative of late stage disease, and/or that the patient is not responsive to chemotherapy. Alternatively, negative samples or lower levels compared to a control (e.g. non-cancerous tissue or negative samples) may also be indicative of progressive disease and shorter overall survival.

In another embodiment, the invention provides methods for determining the presence or absence of endometrial cancer in a subject comprising (a) contacting a sample obtained from the subject with oligonucleotides that hybridize to one or more endometrial cancer marker polynucleotides; and (b) detecting in the sample levels of nucleic acids that hybridize to the polynucleotides relative to a predetermined cut-off value, and therefrom determining the presence or absence of endometrial cancer in the subject. In an embodiment, the endometrial cancer marker polynucleotides encode one or more polypeptides listed in Table 1.

In particular, the invention provides a method wherein chaperonin 10 mRNA is detected by (a) isolating mRNA from a sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction

reagents and nucleic acid primers that hybridize to a polynucleotide encoding chaperonin 10, to produce amplification products; (d) analyzing the amplification products to detect an amount of mRNA encoding chaperonin 10; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and malignant tissue derived using similar nucleic acid primers.

Endometrial cancer marker-positive samples or alternatively higher levels, in particular significantly higher levels of chaperonin 10 polynucleotides in patients compared to a control (e.g. normal or benign) are indicative of endometrial cancer. Negative samples or lower levels compared to a control (e.g. normal or benign) may also be indicative of progressive disease and poor overall survival.

Oligonucleotides or longer fragments derived from an endometrial cancer polynucleotide marker may be used as targets in a microarray. The microarray can be used to simultaneously monitor the expression levels of large numbers of genes and to identify genetic variants, mutations, and polymorphisms. The information from the microarray may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

The preparation, use, and analysis of microarrays are well known to a person skilled in the art. (See, for example, Brennan, T. M. et al. (1995) U.S. Pat. No. 5,474,796; Schena, et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995), PCT Application WO95/251116; Shalon, D. et al. (I 995) PCT application WO95/35505; Heller, R. A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M. J. et al. (1997) U.S. Pat. No. 5,605,662.)

Thus, the invention also includes an array comprising one or more endometrial cancer polynucleotide markers (in particular the markers listed in Table 1), and optionally other markers. The array can be used to assay expression of endometrial polynucleotide markers in the array. The invention allows the quantitation of expression of one or more endometrial markers.

In an embodiment, the array can be used to monitor the time course of expression of one or more endometrial polynucleotide markers in the array. This can occur in various biological contexts such as tumor progression.

The array is also useful for ascertaining differential expression patterns of

endometrial polynucleotide markers, and optionally other markers, in normal and abnormal cells. This may provide a battery of nucleic acids that could serve as molecular targets for diagnosis or therapeutic intervention.

Protein Methods

Binding agents may be used for a variety of diagnostic and assay applications. There are a variety of assay formats known to the skilled artisan for using a binding agent to detect a target molecule in a sample. (For example, see Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988). In general , the presence or absence of an endometrial disease (e.g. cancer) or an endometrium phase in a subject may be determined by (a) contacting a sample from the subject with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined standard or cut-off value.

In particular embodiments of the invention, the binding agent is an antibody. Antibodies specifically reactive with one or more endometrial marker, or derivatives, such as enzyme conjugates or labeled derivatives, may be used to detect one or more endometrial marker in various samples (e.g. biological materials). They may be used as diagnostic or prognostic reagents and they may be used to detect abnormalities in the level of expression of one or more endometrial marker, or abnormalities in the structure, and/or temporal, tissue, cellular, or subcellular location of one or more endometrial marker. Antibodies may also be used to screen potentially therapeutic compounds *in vitro* to determine their effects on disorders (e.g. endometrial cancer) involving one or more endometrial markers, and other conditions. *In vitro* immunoassays may also be used to assess or monitor the efficacy of particular therapies.

In an aspect, the invention provides a diagnostic method for monitoring or diagnosing an endometrial disease (e.g. cancer) in a subject by quantitating one or more endometrial markers in a biological sample from the subject comprising reacting the sample with antibodies specific for one or more endometrial markers, which are directly or indirectly labeled with detectable substances and detecting the detectable substances. In a particular embodiment of the invention, endometrial markers are quantitated or measured.

In an aspect of the invention, a method for detecting an endometrial disease (e.g.

cancer) is provided comprising:

(a) obtaining a sample suspected of containing one or more endometrial markers associated with an endometrial disease;

(b) contacting said sample with antibodies that specifically bind to the endometrial markers under conditions effective to bind the antibodies and form complexes;

(c) measuring the amount of endometrial markers present in the sample by quantitating the amount of the complexes; and

(d) comparing the amount of endometrial markers present in the samples with the amount of endometrial markers in a control, wherein a change or significant difference in the amount of endometrial markers in the sample compared with the amount in the control is indicative of an endometrial disease.

In an embodiment, the invention contemplates a method for monitoring the progression of an endometrial disease (e.g. cancer) in an individual, comprising:

(a) contacting antibodies which bind to one or more endometrial markers with a sample from the individual so as to form complexes comprising the antibodies and one or more endometrial markers in the sample;

(b) determining or detecting the presence or amount of complex formation in the sample;

(c) repeating steps (a) and (b) at a point later in time; and

(d) comparing the result of step (b) with the result of step (c), wherein a difference in the amount of complex formation is indicative of disease, disease stage, and/or progression of the disease in said individual.

The amount of complexes may also be compared to a value representative of the amount of the complexes from an individual not at risk of, or afflicted with, an endometrial disease at different stages. A significant difference in complex formation may be indicative of advanced disease e.g. advanced endometrial cancer, or an unfavourable prognosis.

In embodiments of the methods of the invention, chaperonin 10 is detected in samples and higher levels, in particular significantly higher levels compared to a control (normal or benign) is indicative of endometrial cancer.

In another embodiment, the invention provides methods for determining uterine receptivity of a subject to *in vitro* fertilization comprising (a) contacting a sample obtained from the subject with antibodies that bind to one or more endometrial marker associated with a certain endometrium phase (e.g. secretory phase); and (b) detecting in the sample a level of endometrial marker relative to a predetermined cut-off value, wherein the presence or absence of the endometrial marker as compared to non-receptive controls indicates uterine receptivity. In a particular embodiment, the marker is glutamate receptor subunit zeta 1 or a fragment thereof, and/or macrophage migration inhibitory factor.

Antibodies may be used in any known immunoassays that rely on the binding interaction between antigenic determinants of one or more endometrial marker and the antibodies. Immunoassay procedures for *in vitro* detection of antigens in fluid samples are also well known in the art. [See for example, Paterson et al., Int. J. Can. 37:659 (1986) and Burchell et al., Int. J. Can. 34:763 (1984) for a general description of immunoassay procedures]. Qualitative and/or quantitative determinations of one or more endometrial marker in a sample may be accomplished by competitive or non-competitive immunoassay procedures in either a direct or indirect format. Detection of one or more endometrial marker using antibodies can be done utilizing immunoassays which are run in either the forward, reverse or simultaneous modes. Examples of immunoassays are radioimmunoassays (RIA), enzyme immunoassays (e.g. ELISA), immunofluorescence, immunoprecipitation, latex agglutination, hemagglutination, histochemical tests, and sandwich (immunometric) assays. These terms are well understood by those skilled in the art. A person skilled in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

According to an embodiment of the invention, an immunoassay for detecting one or more endometrial markers in a biological sample comprises contacting binding agents that specifically bind to endometrial markers in the sample under conditions that allow the formation of first complexes comprising a binding agent and endometrial markers and determining the presence or amount of the complexes as a measure of the amount of endometrial markers contained in the sample. In a particular embodiment, the binding agents are labeled differently or are capable of binding to different labels.

Antibodies may be used to detect and quantify one or more endometrial markers in a sample in order to diagnose and treat pathological states. In particular, the antibodies may be used in immunohistochemical analyses, for example, at the cellular and sub-subcellular level, to detect one or more endometrial markers, to localize them to particular endometrial cells and tissues (e.g. tumor cells and tissues), and to specific subcellular locations, and to quantitate the level of expression.

Immunohistochemical methods for the detection of antigens in tissue samples are well known in the art. For example, immunohistochemical methods are described in Taylor, Arch. Pathol. Lab. Med. 102:112 (1978). Briefly, in the context of the present invention, a tissue sample obtained from a subject suspected of having an endometrial-related problem is contacted with antibodies, preferably monoclonal antibodies recognizing one or more endometrial markers. The site at which the antibodies are bound is determined by selective staining of the sample by standard immunohistochemical procedures. The same procedure may be repeated on the same sample using other antibodies that recognize one or more endometrial markers. Alternatively, a sample may be contacted with antibodies against one or more endometrial markers simultaneously, provided that the antibodies are labeled differently or are able to bind to a different label. The tissue sample may be normal endometrial tissue, a cancer tissue or a benign tissue.

Antibodies specific for one or more endometrial marker may be labelled with a detectable substance and localised in biological samples based upon the presence of the detectable substance. Examples of detectable substances include, but are not limited to, the following: radioisotopes (e.g., ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{131}I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), luminescent labels such as luminol; enzymatic labels (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase), biotinyl groups (which can be detected by marked avidin e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods), predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached via spacer arms of various lengths to reduce potential steric hindrance. Antibodies may also be coupled to electron dense substances, such as ferritin or colloidal gold, which are readily visualised by electron microscopy.

One of the ways an antibody can be detectably labeled is to link it directly to an enzyme. The enzyme when later exposed to its substrate will produce a product that can be detected. Examples of detectable substances that are enzymes are horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase, acetylcholinesterase, malate dehydrogenase, ribonuclease, urease, catalase, glucose-6-phosphate, staphylococcal nuclease, delta-5-steriod isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, triose phosphate isomerase, asparaginase, glucose oxidase, and acetylcholine esterase.

For increased sensitivity in an immunoassay system a fluorescence-emitting metal atom such as Eu (europium) and other lanthanides can be used. These can be attached to the desired molecule by means of metal-chelating groups such as DTPA or EDTA.

A bioluminescent compound may also be used as a detectable substance. Bioluminescence is a type of chemiluminescence found in biological systems where a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent molecule is determined by detecting the presence of luminescence. Examples of bioluminescent detectable substances are luciferin, luciferase and aequorin.

Indirect methods may also be employed in which the primary antigen-antibody reaction is amplified by the introduction of a second antibody, having specificity for the antibody reactive against one or more endometrial markers. By way of example, if the antibody having specificity against one or more endometrial markers is a rabbit IgG antibody, the second antibody may be goat anti-rabbit gamma-globulin labelled with a detectable substance as described herein.

Methods for conjugating or labelling the antibodies discussed above may be readily accomplished by one of ordinary skill in the art. (See for example Inman, Methods In Enzymology, Vol. 34, Affinity Techniques, Enzyme Purification: Part B, Jakoby and Wichek (eds.), Academic Press, New York, p. 30, 1974; and Wilchek and Bayer, "The Avidin-Biotin Complex in Bioanalytical Applications,"Anal. Biochem. 171:1-32, 1988 re methods for conjugating or labelling the antibodies with enzyme or ligand binding partner).

Cytochemical techniques known in the art for localizing antigens using light and electron microscopy may be used to detect a one or more endometrial markers.

Generally, antibodies may be labeled with detectable substances and one or more endometrial markers may be localised in tissues and cells based upon the presence of the detectable substances.

In the context of the methods of the invention, the sample, binding agents (e.g. antibodies specific for one or more endometrial markers), or one or more endometrial markers may be immobilized on a carrier or support. Examples of suitable carriers or supports are agarose, cellulose, nitrocellulose, dextran, Sephadex, Sepharose, liposomes, carboxymethyl cellulose, polyacrylamides, polystyrene, gabbros, filter paper, magnetite, ion-exchange resin, plastic film, plastic tube, glass, polyamine-methyl vinyl-ether-maleic acid copolymer, amino acid copolymer, ethylene-maleic acid copolymer, nylon, silk, etc. The support material may have any possible configuration including spherical (e.g. bead), cylindrical (e.g. inside surface of a test tube or well, or the external surface of a rod), or flat (e.g. sheet, test strip). Thus, the carrier may be in the shape of, for example, a tube, test plate, well, beads, disc, sphere, etc. The immobilized antibody may be prepared by reacting the material with a suitable insoluble carrier using known chemical or physical methods, for example, cyanogen bromide coupling. An antibody may be indirectly immobilized using a second antibody specific for the antibody. For example, mouse antibody specific for an endometrial marker may be immobilized using sheep anti-mouse IgG Fc fragment specific antibody coated on the carrier or support.

Where a radioactive label is used as a detectable substance, one or more endometrial marker may be localized by radioautography. The results of radioautography may be quantitated by determining the density of particles in the radioautographs by various optical methods, or by counting the grains.

Time-resolved fluorometry may be used to detect a signal. For example, the method described in Christopoulos TK and Diamandis EP Anal Chem 1992:64:342-346 may be used with a conventional time-resolved fluorometer.

In accordance with an embodiment of the invention, a method is provided wherein one or more endometrial marker antibodies are directly or indirectly labelled with enzymes, substrates for the enzymes are added wherein the substrates are selected so that the substrates, or a reaction product of an enzyme and substrate, form fluorescent complexes with a lanthanide metal (e.g. europium, terbium, samarium, and dysprosium, preferably europium and terbium). A lanthanide metal is added and one or more

endometrial cancer markers are quantitated in the sample by measuring fluorescence of the fluorescent complexes. Enzymes are selected based on the ability of a substrate of the enzyme, or a reaction product of the enzyme and substrate, to complex with lanthanide metals such as europium and terbium. Suitable enzymes and substrates that provide fluorescent complexes are described in U.S. Patent No. 5,3112,922 to Diamandis. Examples of suitable enzymes include alkaline phosphatase and β-galactosidase. Preferably, the enzyme is alkaline phosphatase.

Examples of enzymes and substrates for enzymes that provide such fluorescent complexes are described in U.S. Patent No. 5,312,922 to Diamandis. By way of example, when the antibody is directly or indirectly labelled with alkaline phosphatase the substrate employed in the method may be 4-methylumbelliferyl phosphate, 5-fluorosalicyl phosphate, or diflunisal phosphate. The fluorescence intensity of the complexes is typically measured using a time-resolved fluorometer e.g. a CyberFluor 615 Imunoanalyzer (Nordion International, Kanata, Ontario).

One or more endometrial marker antibodies may also be indirectly labelled with an enzyme. For example, the antibodies may be conjugated to one partner of a ligand binding pair, and the enzyme may be coupled to the other partner of the ligand binding pair. Representative examples include avidin-biotin, and riboflavin-riboflavin binding protein. In an embodiment, the antibodies are biotinylated, and the enzyme is coupled to streptavidin. In another embodiment, an antibody specific for endometrial marker antibody is labeled with an enzyme.

In accordance with an embodiment, the present invention provides means for determining one or more endometrial markers in a sample by measuring one or more endometrial markers by immunoassay. It will be evident to a skilled artisan that a variety of immunoassay methods can be used to measure one or more endometrial markers. In general, an immunoassay method may be competitive or noncompetitive. Competitive methods typically employ an immobilized or immobilizable antibody to one or more endometrial marker and a labeled form of one or more endometrial marker. Sample endometrial markers and labeled endometrial markers compete for binding to antibodies to endometrial markers. After separation of the resulting labeled endometrial markers that have become bound to antibodies (bound fraction) from that which has remained unbound (unbound fraction), the amount of the label in either bound or unbound fraction

is measured and may be correlated with the amount of endometrial markers in the test sample in any conventional manner, e.g., by comparison to a standard curve.

In an aspect, a non-competitive method is used for the determination of one or more endometrial markers, with the most common method being the "sandwich" method. In this assay, two antibodies to endometrial markers are employed. One of the antibodies to endometrial markers is directly or indirectly labeled (sometimes referred to as the "detection antibody") and the other is immobilized or immobilizable (sometimes referred to as the "capture antibody"). The capture and detection antibodies can be contacted simultaneously or sequentially with the test sample. Sequential methods can be accomplished by incubating the capture antibody with the sample, and adding the detection antibody at a predetermined time thereafter (sometimes referred to as the "forward" method); or the detection antibody can be incubated with the sample first and then the capture antibody added (sometimes referred to as the "reverse" method). After the necessary incubation(s) have occurred, to complete the assay, the capture antibody is separated from the liquid test mixture, and the label is measured in at least a portion of the separated capture antibody phase or the remainder of the liquid test mixture. Generally it is measured in the capture antibody phase since it comprises endometrial cancer markers bound by ("sandwiched" between) the capture and detection antibodies. In an embodiment, the label may be measured without separating the capture antibodies and liquid test mixture.

In a typical two-site immunometric assay for endometrial markers, one or both of the capture and detection antibodies are polyclonal antibodies or one or both of the capture and detection antibodies are monoclonal antibodies (i.e. polyclonal/polyclonal, monoclonal/monoclonal, or monoclonal/polyclonal). The label used in the detection antibody can be selected from any of those known conventionally in the art. The label may be an enzyme or a chemiluminescent moiety, but it can also be a radioactive isotope, a fluorophor, a detectable ligand (e.g., detectable by a secondary binding by a labeled binding partner for the ligand), and the like. In a particular aspect, the antibody is labelled with an enzyme which is detected by adding a substrate that is selected so that a reaction product of the enzyme and substrate forms fluorescent complexes. The capture antibody may be selected so that it provides a means for being separated from the remainder of the test mixture. Accordingly, the capture antibody can be introduced to the

assay in an already immobilized or insoluble form, or can be in an immobilizable form, that is, a form which enables immobilization to be accomplished subsequent to introduction of the capture antibody to the assay. An immobilized capture antibody may comprise an antibody covalently or noncovalently attached to a solid phase such as a magnetic particle, a latex particle, a microtiter plate well, a bead, a cuvette, or other reaction vessel. An example of an immobilizable capture antibody is antibody which has been chemically modified with a ligand moiety, e.g., a hapten, biotin, or the like, and which can be subsequently immobilized by contact with an immobilized form of a binding partner for the ligand, e.g., an antibody, avidin, or the like. In an embodiment, the capture antibody may be immobilized using a species specific antibody for the capture antibody that is bound to the solid phase.

The above-described immunoassay methods and formats are intended to be exemplary and are not limiting.

Computer Systems

Computer readable media comprising one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers (e.g. markers of endometrial cancer) is also provided. "Computer readable media" refers to any medium that can be read and accessed directly by a computer, including but not limited to magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. Thus, the invention contemplates computer readable medium having recorded thereon markers identified for patients and controls.

"Recorded" refers to a process for storing information on computer readable medium. The skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising information on one or more endometrial markers, and optionally other markers.

A variety of data processor programs and formats can be used to store information on one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and other markers on computer readable medium. For example, the information can be represented in a word processing text file, formatted in

commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. Any number of dataprocessor structuring formats (e.g., text file or database) may be adapted in order to obtain computer readable medium having recorded thereon the marker information.

By providing the marker information in computer readable form, one can routinely access the information for a variety of purposes. For example, one skilled in the art can use the information in computer readable form to compare marker information obtained during or following therapy with the information stored within the data storage means.

The invention provides a medium for holding instructions for performing a method for determining uterine endometrial receptivity of a patient, or whether a patient has an endometrial disease (e.g. endometrial cancer) or a pre-disposition to an endometrial disease (e.g. cancer), comprising determining the presence or absence of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers, and based on the presence or absence of the one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers, determining uterine endometrial receptivity, has an endometrial disease (e.g. cancer) or a pre-disposition to an endometrial disease (e.g. cancer), and optionally recommending a procedure or treatment.

The invention also provides in an electronic system and/or in a network, a method for determining uterine endometrial receptivity of a patient, whether a subject has an endometrial disease (e.g. cancer) or a pre-disposition to an endometrial disease (e.g. cancer), comprising determining the presence or absence of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers (e.g. cancer markers), and based on the presence or absence of the one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers, determining the uterine endometrial receptivity of the patient, whether the subject has an endometrial disease (e.g. cancer) or a pre-disposition to an endometrial disease (e.g. cancer), and optionally recommending a procedure or treatment.

The invention further provides in a network, a method for determining whether a subject is receptive to *in vitro* fertilization, has an endometrial disease (e.g. cancer) or a pre-disposition to an endometrial disease (e.g. cancer) comprising: (a) receiving phenotypic information on the subject and information on one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers associated with samples from the subject; (b) acquiring information from the network corresponding to the one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers; and (c) based on the phenotypic information and information on the one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers, determining whether the subject is receptive to *in vitro* fertilization, has an endometrial disease (e.g. cancer) or a pre-disposition to an endometrial disease (e.g. cancer); and (d) optionally recommending a procedure or treatment.

The invention still further provides a system for identifying selected records that identify a diseased endometrial cell or tissue (e.g. cancer cell or tissue) or an endometrium phase. A system of the invention generally comprises a digital computer; a database server coupled to the computer; a database coupled to the database server having data stored therein, the data comprising records of data comprising one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other endometrial markers, and a code mechanism for applying queries based upon a desired selection criteria to the data file in the database to produce reports of records which match the desired selection criteria.

In an aspect of the invention a method is provided for detecting endometrial cancer tissue or cells using a computer having a processor, memory, display, and input/output devices, the method comprising the steps of:

(a) creating records of one or more endometrial cancer markers, and/or polynucleotides encoding one or more endometrial cancer markers, and optionally other markers of cancer identified in a sample suspected of containing endometrial cancer cells or tissue;

(b) providing a database comprising records of data comprising one or more endometrial cancer markers, and/or polynucleotides encoding one or more

endometrial cancer markers, and optionally other markers of cancer; and

(c) using a code mechanism for applying queries based upon a desired selection criteria to the data file in the database to produce reports of records of step (a) which provide a match of the desired selection criteria of the database of step (b) the presence of a match being a positive indication that the markers of step (a) have been isolated from cells or tissue that are endometrial cancer cells or tissue.

The invention contemplates a business method for determining whether a subject is receptive to *in vitro* fertilization, has an endometrial disease (e.g. cancer) or a pre-disposition to endometrial cancer comprising: (a) receiving phenotypic information on the subject and information on one or more endometrial markers, and/or polynucleotides encoding the markers, and optionally other markers, associated with samples from the subject; (b) acquiring information from a network corresponding to one or more endometrial markers, and/or polynucleotides encoding the markers, and optionally other markers; and (c) based on the phenotypic information, information on one or more endometrial markers, and/or polynucleotides encoding the markers, and optionally other markers, and acquired information, determining whether the subject is receptive to in vitro fertilization, has an endometrial disease (e.g. cancer) or a pre-disposition to an endometrial disease (e.g. cancer); and (d) optionally recommending a procedure or treatment.

In an aspect of the invention, the computer systems, components, and methods described herein are used to monitor disease or determine the stage of disease, or determine uterine endometrial receptivity.

Imaging Methods

Binding agents, in particular antibodies, specific for one or more endometrial markers may also be used in imaging methodologies in the management of an endometrial disease or determining uterine endometrial receptivity.

In an aspect, the invention provides a method for imaging tumors associated with one or more endometrial cancer markers.

The invention also contemplates imaging methods described herein using multiple markers for an endometrial disease or endometrium phase. Preferably each agent is labeled so that it can be distinguished during the imaging.

In an embodiment the method is an *in vivo* method and a subject or patient is administered one or more agents that carry an imaging label and that are capable of targeting or binding to one or more endometrial markers. The agent is allowed to incubate *in vivo* and bind to the endometrial markers associated with a diseased cell or tissue, (e.g. an endometrial tumor). The presence of the label is localized to the diseased endometrial cell or tissue, and the localized label is detected using imaging devices known to those skilled in the art.

The agent may be an antibody or chemical entity that recognizes the endometrial markers. In an aspect of the invention the agent is a polyclonal antibody or monoclonal antibody, or fragments thereof, or constructs thereof including but not limited to, single chain antibodies, bifunctional antibodies, molecular recognition units, and peptides or entities that mimic peptides. The antibodies specific for the endometrial markers used in the methods of the invention may be obtained from scientific or commercial sources, or isolated native endometrial markers or recombinant endometrial markers may be utilized to prepare antibodies etc. as described herein.

An agent may be a peptide that mimics the epitope for an antibody specific for an endometrial marker and binds to the marker. The peptide may be produced on a commercial synthesizer using conventional solid phase chemistry. By way of example, a peptide may be prepared that includes either tyrosine, lysine, or phenylalanine to which N_2S_2 chelate is complexed (See U.S. Patent No. 4,897,255). An anti-endocrine marker peptide conjugate is then combined with a radiolabel (e.g. sodium ^{99m}Tc pertechnetate or sodium ^{188}Re perrhenate) and it may be used to locate an endometrial marker producing cell or tissue (e.g. tumor).

The agent carries a label to image the endometrial markers. The agent may be labelled for use in radionuclide imaging. In particular, the agent may be directly or indirectly labelled with a radioisotope. Examples of radioisotopes that may be used in the present invention are the following: ^{277}Ac, ^{211}At, ^{128}Ba, ^{131}Ba, ^{7}Be, ^{204}Bi, ^{205}Bi, ^{206}Bi, ^{76}Br, ^{77}Br, ^{82}Br, ^{109}Cd, ^{47}Ca, ^{11}C, ^{14}C, ^{36}Cl, ^{48}Cr, ^{51}Cr, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{165}Dy, ^{155}Eu, ^{18}F, ^{153}Gd, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{72}Ga, ^{198}Au, ^{3}H, ^{166}Ho, ^{111}In, ^{113m}In, ^{115m}In, ^{123}I, ^{125}I, ^{131}I, ^{189}Ir, ^{191m}Ir, ^{192}Ir, ^{194}Ir, ^{52}Fe, ^{55}Fe, ^{59}Fe, ^{177}Lu, ^{15}O, $^{191m-191}$Os, ^{109}Pd, ^{32}P, ^{33}P, ^{42}K, ^{226}Ra, ^{186}Re, ^{188}Re, ^{82m}Rb, ^{153}Sm, ^{46}Sc, ^{47}Sc, ^{72}Se, ^{75}Se, ^{105}Ag, ^{22}Na, ^{24}Na, ^{89}Sr, ^{35}S, ^{38}S, ^{177}Ta, ^{96}Tc, ^{99m}Tc, ^{201}Tl, ^{202}Tl, ^{113}Sn, ^{117m}Sn, ^{121}Sn, ^{166}Yb, ^{169}Yb, ^{175}Yb, ^{88}Y, ^{90}Y, ^{62}Zn and ^{65}Zn.

Preferably the radioisotope is ^{131}I, ^{125}I, ^{123}I, ^{111}I, ^{99m}Tc, ^{90}Y, ^{186}Re, ^{188}Re, ^{32}P, ^{153}Sm, ^{67}Ga, ^{201}Tl ^{77}Br, or ^{18}F, and is imaged with a photoscanning device.

Procedures for labeling biological agents with the radioactive isotopes are generally known in the art. U.S. Pat. No. 4,302,438 describes tritium labeling procedures. Procedures for iodinating, tritium labeling, and ^{35} S labeling especially adapted for murine monoclonal antibodies are described by Goding, J. W. (supra, pp 124-126) and the references cited therein. Other procedures for iodinating biological agents, such as antibodies, binding portions thereof, probes, or ligands, are described in the scientific literature (see Hunter and Greenwood, Nature 144:945 (1962), David et al., Biochemistry 13:1014-1021 (1974), and U.S. Pat. Nos. 3,867,517 and 4,376,110). Iodinating procedures for agents are described by Greenwood, F. et al., Biochem. J. 89:114-123 (1963); Marchalonis, J., Biochem. J. 113:299-305 (1969); and Morrison, M. et al., Immunochemistry, 289-297 (1971). ^{99m} Tc-labeling procedures are described by Rhodes, B. et al. in Burchiel, S. et al. (eds.), Tumor Imaging: The Radioimmunochemical Detection of Cancer, New York: Masson 111-123 (1982) and the references cited therein. Labelling of antibodies or fragments with technetium-99m are also described for example in U.S. Pat. No. 5,317,091, U.S. Pat. No. 4,478,815, U.S. Pat. No. 4,478,818, U.S. Pat. No. 4,472,371, U.S. Pat. No. Re 32,417, and U.S. Pat. No. 4,311,688. Procedures suitable for ^{111} In-labeling biological agents are described by Hnatowich, D. J. et al., J. Immul. Methods, 65:147-157 (1983), Hnatowich, D. et al., J. Applied Radiation, 35:554-557 (1984), and Buckley, R. G. et al., F.E.B.S. 166:202-204 (1984).

An agent may also be labeled with a paramagnetic isotope for purposes of an *in vivo* method of the invention. Examples of elements that are useful in magnetic resonance imaging include gadolinium, terbium, tin, iron, or isotopes thereof. (See, for example, Schaefer et al., (1989) JACC 14, 472-480; Shreve et al., (1986) Magn. Reson. Med. 3, 336-340; Wolf, G L., (1984) Physiol. Chem. Phys. Med. NMR 16, 93-95; Wesbey et al., (1984) Physiol. Chem. Phys. Med. NMR 16, 145-155; Runge et al., (1984) Invest. Radiol. 19, 408-415 for discussions on *in vivo* nuclear magnetic resonance imaging.)

In the case of a radiolabeled agent, the agent may be administered to the patient, it is localized to the cell or tissue (e.g. tumor) having an endometrial marker with which the agent binds, and is detected or "imaged" *in vivo* using known techniques such as

radionuclear scanning using e.g., a gamma camera or emission tomography. [See for example, A. R. Bradwell et al., "Developments in Antibody Imaging", Monoclonal Antibodies for Cancer Detection and Therapy, R. W. Baldwin et al., (eds.), pp. 65-85 (Academic Press 1985)]. A positron emission transaxial tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can also be used where the radiolabel emits positrons (e.g., ^{11}C, ^{18}F, ^{15}O, and ^{13}N).

Whole body imaging techniques using radioisotope labeled agents can be used for locating diseased cells and tissues (e.g. primary tumors and tumors which have metastasized). Antibodies specific for endometrial markers, or fragments thereof having the same epitope specificity, are bound to a suitable radioisotope, or a combination thereof, and administered parenterally. For endometrial cancer, administration preferably is intravenous. The bio-distribution of the label can be monitored by scintigraphy, and accumulations of the label are related to the presence of endometrial cancer cells. Whole body imaging techniques are described in U.S. Pat. Nos. 4,036,945 and 4,311,688. Other examples of agents useful for diagnosis and therapeutic use that can be coupled to antibodies and antibody fragments include metallothionein and fragments (see, U.S. Pat. No. 4,732,864). These agents are useful in diagnosis staging and visualization of cancer, in particular endometrial cancer, so that surgical and/or radiation treatment protocols can be used more efficiently.

An imaging agent may carry a bioluminescent or chemiluminescent label. Such labels include polypeptides known to be fluorescent, bioluminescent or chemiluminescent, or, that act as enzymes on a specific substrate (reagent), or can generate a fluorescent, bioluminescent or chemiluminescent molecule. Examples of bioluminescent or chemiluminescent labels include luciferases, aequorin, obelin, mnemiopsin, berovin, a phenanthridinium ester, and variations thereof and combinations thereof. A substrate for the bioluminescent or chemiluminescent polypeptide may also be utilized in a method of the invention. For example, the chemiluminescent polypeptide can be luciferase and the reagent luciferin. A substrate for a bioluminescent or chemiluminescent label can be administered before, at the same time (e.g., in the same formulation), or after administration of the agent.

An imaging agent may comprise a paramagnetic compound, such as a polypeptide chelated to a metal, e.g., a metalloporphyrin. The paramagnetic compound

may also comprise a monocrystalline nanoparticle, e.g., a nanoparticle comprising a lanthanide (e.g., Gd) or iron oxide; or, a metal ion comprising a lanthanide. "Lanthanides" refers to elements of atomic numbers 58 to 70, a transition metal of atomic numbers 21 to 29, 42 or 44, a Gd(III), a Mn(II), or an element comprising an Fe element. Paramagnetic compounds can also comprise a neodymium iron oxide ($NdFeO_3$) or a dysprosium iron oxide ($DyFeO_3$). Examples of elements that are useful in magnetic resonance imaging include gadolinium, terbium, tin, iron, or isotopes thereof. (See, for example, Schaefer et al., (1989) JACC 14, 472-480; Shreve et al., (1986) Magn. Reson. Med. 3, 336-340; Wolf, G L., (1984) Physiol. Chem. Phys. Med. NMR 16, 93-95; Wesbey et al., (1984) Physiol. Chem. Phys. Med. NMR 16, 145-155; Runge et al., (1984) Invest. Radiol. 19, 408-415 for discussions on *in vivo* nuclear magnetic resonance imaging.)

An image can be generated in a method of the invention by computer assisted tomography (CAT), magnetic resonance spectroscopy (MRS) image, magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), or bioluminescence imaging (BLI) or equivalent.

Computer assisted tomography (CAT) and computerized axial tomography (CAT) systems and devices well known in the art can be utilized in the practice of the present invention. (See, for example, U.S. Patent Nos. 6,151,377; 5,946,371; 5,446,799; 5,406,479; 5,208,581; 5,109,397). The invention may also utilize animal imaging modalities, such as MicroCAT.TM. (ImTek, Inc.).

Magnetic resonance imaging (MRI) systems and devices well known in the art can be utilized in the practice of the present invention. In magnetic resonance methods and devices, a static magnetic field is applied to a tissue or a body in order to define an equilibrium axis of magnetic alignment in a region of interest. A radio frequency field is then applied to the region in a direction orthogonal to the static magnetic field direction to excite magnetic resonance in the region. The resulting radio frequency signals are then detected and processed, and the exciting radio frequency field is applied. The resulting signals are detected by radio-frequency coils that are placed adjacent to the tissue or area of the body of interest. (For a description of MRI methods and devices see, for example, U.S. Patent Nos. 6,151,377; 6,144,202; 6,128,522; 6,127,825; 6,121,775; 6,119,032; 6,115,446; 6,111,410; 602,891; 5,555,251; 5,455,512; 5,450,010; 5,378,987; 5,214,382;

5,031,624; 5,207,222; 4,985,678; 4,906,931; 4,558,279). MRI and supporting devices are commercially available for example, from Bruker Medical GMBH; Caprius; Esaote Biomedica; Fonar; GE Medical Systems (GEMS); Hitachi Medical Systems America; Intermagnetics General Corporation; Lunar Corp.; MagneVu; Marconi Medicals; Philips Medical Systems; Shimadzu; Siemens; Toshiba America Medical Systems; including imaging systems, by, e.g., Silicon Graphics. The invention may also utilize animal imaging modalities such as micro-MRIs.

Positron emission tomography imaging (PET) systems and devices well known in the art can be utilized in the practice of the present invention. For example, a method of the invention may use the system designated Pet VI located at Brookhaven National Laboratory. For descriptions of PET systems and devices see, for example, U.S. Pat. Nos. 6,151,377; 6,072,177; 5,900,636; 5,608,221; 5,532,489; 5,272,343; 5,103,098. Animal imaging modalities such as micro-PETs (Corcorde Microsystems, Inc.) can also be used in the invention.

Single-photon emission computed tomography (SPECT) systems and devices well known in the art can be utilized in the practice of the present invention. (See, for example, U.S. Patents. Nos. 6,115,446; 6,072,177; 5,608,221; 5,600,145; 5,210,421; 5,103,098.) The methods of the invention may also utilize animal imaging modalities, such as micro-SPECTs.

Bioluminescence imaging includes bioluminescence, fluorescence or chemiluminescence or other photon detection systems and devices that are capable of detecting bioluminescence, fluorescence or chemiluminescence. Sensitive photon detection systems can be used to detect bioluminescent and fluorescent proteins externally; see, for example, Contag (2000) Neoplasia 2:41-52; Zhang (1994) Clin. Exp. Metastasis 12:87-92. The methods of the invention can be practiced using any such photon detection device, or variation or equivalent thereof, or in conjunction with any known photon detection methodology, including visual imaging. By way of example, an intensified charge-coupled device (ICCD) camera coupled to an image processor may be used in the present invention. (See, e.g., U.S. Pat. No. 5,650,135). Photon detection devices are also commercially available from Xenogen, Hamamatsue.

Screening Methods

The invention also contemplates methods for evaluating test agents or

compounds for their ability to inhibit an endometrial disease (e.g. cancer), potentially contribute to an endometrial disease (e.g. cancer), or inhibit or enhance an endometrium phase. Test agents and compounds include but are not limited to peptides such as soluble peptides including Ig-tailed fusion peptides, members of random peptide libraries and combinatorial chemistry-derived molecular libraries made of D- and/or L-configuration amino acids, phosphopeptides (including members of random or partially degenerate, directed phosphopeptide libraries), antibodies [e.g. polyclonal, monoclonal, humanized, anti-idiotypic, chimeric, single chain antibodies, fragments, (e.g. Fab, $F(ab)_2$, and Fab expression library fragments, and epitope-binding fragments thereof)], and small organic or inorganic molecules. The agents or compounds may be endogenous physiological compounds or natural or synthetic compounds.

The invention provides a method for assessing the potential efficacy of a test agent for inhibiting an endometrial disease (e.g. cancer) in a patient, the method comprising comparing:

(a) levels of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers in a first sample obtained from a patient and exposed to the test agent; and

(b) levels of one or more endometrial markers and/or polynucleotides encoding one or more endometrial markers, and optionally other markers in a second sample obtained from the patient, wherein the sample is not exposed to the test agent, wherein a significant difference in the levels of expression of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally the other markers, in the first sample, relative to the second sample, is an indication that the test agent is potentially efficacious for inhibiting an endometrial disease (e.g. cancer) in the patient.

The first and second samples may be portions of a single sample obtained from a patient or portions of pooled samples obtained from a patient.

In an aspect, the invention provides a method of selecting an agent for inhibiting an endometrial disease (e.g. cancer) in a patient comprising:

(a) obtaining a sample from the patient;

(b) separately maintaining aliquots of the sample in the presence of a plurality

of test agents;

(c) comparing one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers, in each of the aliquots; and

(d) selecting one of the test agents which alters the levels of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers in the aliquot containing that test agent, relative to other test agents.

In a further aspect, the invention provides a method of selecting an agent for inhibiting or enhancing an endometrium phase in a patient comprising:

(a) obtaining a sample of endometrium in a selected phase (e.g. secretory or proliferative phase);

(b) separately maintaining aliquots of the sample in the presence of a plurality of test agents;

(c) comparing one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers, in each of the aliquots; and

(d) selecting one of the test agents which alters the levels of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers in the aliquot containing that test agent, relative to other test agents.

Still another aspect of the present invention provides a method of conducting a drug discovery business comprising:

(a) providing one or more methods or assay systems for identifying agents that inhibit an endometrial disease (e.g. endometrial cancer) or affect an endometrium phase in a patient;

(b) conducting therapeutic profiling of agents identified in step (a), or further analogs thereof, for efficacy and toxicity in animals; and

(c) formulating a pharmaceutical preparation including one or more agents identified in step (b) as having an acceptable therapeutic profile.

In certain embodiments, the subject method can also include a step of establishing a distribution system for distributing the pharmaceutical preparation for sale,

and may optionally include establishing a sales group for marketing the pharmaceutical preparation.

The invention also contemplates a method of assessing the potential of a test compound to contribute to an endometrial disease (e.g. endometrial cancer) comprising:

(a) maintaining separate aliquots of cells or tissues from a patient with an endometrial disease (e.g. cancer) in the presence and absence of the test compound; and

(b) comparing one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers, and optionally other markers in each of the aliquots.

A significant difference between the levels of the markers in the aliquot maintained in the presence of (or exposed to) the test compound relative to the aliquot maintained in the absence of the test compound, indicates that the test compound possesses the potential to contribute to an endometrial disease (e.g. endometrial cancer).

Kits

The invention also contemplates kits for carrying out the methods of the invention. Kits may typically comprise two or more components required for performing a diagnostic assay. Components include but are not limited to compounds, reagents, containers, and/or equipment.

The methods described herein may be performed by utilizing pre-packaged diagnostic kits comprising one or more specific endometrial marker polynucleotide or antibody described herein, which may be conveniently used, e.g., in clinical settings to screen and diagnose patients and to screen and identify those individuals exhibiting a predisposition to developing an endometrial disease.

In an embodiment, a container with a kit comprises a binding agent as described herein. By way of example, the kit may contain antibodies or antibody fragments which bind specifically to epitopes of one or more endometrial markers and optionally other markers, antibodies against the antibodies labelled with an enzyme; and a substrate for the enzyme. The kit may also contain microtiter plate wells, standards, assay diluent, wash buffer, adhesive plate covers, and/or instructions for carrying out a method of the invention using the kit.

In an aspect of the invention, the kit includes antibodies or fragments of antibodies which bind specifically to an epitope of one or more protein listed in Table 1, and means for detecting binding of the antibodies to their epitope associated with tumor cells, either as concentrates (including lyophilized compositions), which may be further diluted prior to use or at the concentration of use, where the vials may include one or more dosages. Where the kits are intended for *in vivo* use, single dosages may be provided in sterilized containers, having the desired amount and concentration of agents. Containers that provide a formulation for direct use, usually do not require other reagents, as for example, where the kit contains a radiolabelled antibody preparation for *in vivo* imaging.

A kit may be designed to detect the level of polynucleotides encoding one or more endometrial polynucleotide markers in a sample. In an embodiment, the polynucleotides encode one or more polynucleotides encoding a polypeptide listed in Table 1. Such kits generally comprise at least one oligonucleotide probe or primer, as described herein, that hybridizes to a polynucleotide encoding one or more endometrial cancer markers. Such an oligonucleotide may be used, for example, within a PCR or hybridization procedure. Additional components that may be present within the kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate detection of a polynucleotide encoding one or more endometrial cancer markers.

The reagents suitable for applying the screening methods of the invention to evaluate compounds may be packaged into convenient kits described herein providing the necessary materials packaged into suitable containers.

The invention relates to a kit for assessing the suitability of each of a plurality of test compounds for inhibiting an endometrial disease (e.g. endometrial cancer) in a patient. The kit comprises reagents for assessing one or more endometrial markers or polynucleotides encoding same, and optionally a plurality of test agents or compounds.

The invention contemplates a kit for assessing the presence of endometrial cells, wherein the kit comprises antibodies specific for one or more endometrial markers, or primers or probes for polynucleotides encoding same, and optionally probes, primers or antibodies specific for other markers associated with an endometrial disease (e.g. cancer).

Additionally the invention provides a kit for assessing the potential of a test compound to contribute to an endometrial disease (e.g. cancer). The kit comprises endometrial diseased cells (e.g. cancer cells) and reagents for assessing one or more endometrial markers, polynucleotides encoding same, and optionally other markers associated with an endometrial disease.

Therapeutic Applications

One or more endometrial markers may be targets for immunotherapy. Immunotherapeutic methods include the use of antibody therapy, *in vivo* vaccines, and *ex vivo* immunotherapy approaches.

In one aspect, the invention provides one or more endometrial marker antibodies that may be used systemically to treat an endometrial disease associated with the marker. In particular, the endometrial disease is endometrial cancer and one or more endometrial marker antibodies may be used systemically to treat endometrial cancer. Preferably antibodies are used that target the tumor cells but not the surrounding non-tumor cells and tissue.

Thus, the invention provides a method of treating a patient susceptible to, or having a disease (e.g. cancer) that expresses one or more endometrial marker, comprising administering to the patient an effective amount of an antibody that binds specifically to one or more endometrial marker.

In another aspect, the invention provides a method of inhibiting the growth of tumor cells expressing one or more endometrial cancer markers, comprising administering to a patient an antibody which binds specifically to one or more endometrial cancer markers in an amount effective to inhibit growth of the tumor cells.

One or more endometrial marker antibodies may also be used in a method for selectively inhibiting the growth of or killing a cell expressing one or more endometrial marker (e.g. tumor cell expressing one or more endometrial cancer marker) comprising reacting one or more endometrial marker antibody immunoconjugate or immunotoxin with the cell in an amount sufficient to inhibit the growth of or kill the cell.

By way of example, unconjugated antibodies to endometrial cancer markers may be introduced into a patient such that the antibodies bind to endometrial cancer marker expressing cancer cells and mediate growth inhibition of such cells (including the destruction thereof), and the tumor, by mechanisms which may include complement-

mediated cytolysis, antibody-dependent cellular cytotoxicity, altering the physiologic function of one or more endometrial cancer markers, and/or the inhibition of ligand binding or signal transduction pathways. In addition to unconjugated antibodies to endometrial cancer markers, one or more endometrial cancer marker antibodies conjugated to therapeutic agents (e.g. immunoconjugates) may also be used therapeutically to deliver the agent directly to one or more endometrial cancer marker expressing tumor cells and thereby destroy the tumor. Examples of such agents include abrin, ricin A, *Pseudomonas* exotoxin, or diphtheria toxin; proteins such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator; and biological response modifiers such as lymphokines, interleukin-1, interleukin-2, interleukin-6, granulocyte macrophage colony stimulating factor, granulocyte colony stimulating factor, or other growth factors.

Cancer immunotherapy using one or more endometrial cancer marker antibodies may utilize the various approaches that have been successfully employed for cancers, including but not limited to colon cancer (Arlen et al., 1998, Crit Rev Immunol 18: 133-138), multiple myeloma (Ozaki et al., 1997, Blood 90: 3179-3186; Tsunenati et al., 1997, Blood 90: 2437-2444), gastric cancer (Kasprzyk et al., 1992, Cancer Res 52: 2771-2776), B-cell lymphoma (Funakoshi et al., 1996, J Immunther Emphasis Tumor Immunol 19: 93-101), leukemia (Zhong et al., 1996, Leuk Res 20: 581-589), colorectal cancer (Moun et al., 1994, Cancer Res 54: 6160-6166); Velders et al., 1995, Cancer Res 55: 4398-4403), and breast cancer (Shepard et al., 1991, J Clin Immunol 11: 117-127).

In the practice of a method of the invention, endometrial cancer marker antibodies capable of inhibiting the growth of cancer cells expressing endometrial cancer markers are administered in a therapeutically effective amount to cancer patients whose tumors express or overexpress one or more endometrial cancer markers. The invention may provide a specific, effective and long-needed treatment for endometrial cancer. The antibody therapy methods of the invention may be combined with other therapies including chemotherapy and radiation.

Patients may be evaluated for the presence and level of expression or overexpression of one or more endometrial markers in diseased cells and tissues (e.g. tumors), in particular using immunohistochemical assessments of tissue, quantitative imaging as described herein, or other techniques capable of reliably indicating the

presence and degree of expression of one or more endometrial markers. Immunohistochemical analysis of tumor biopsies or surgical specimens may be employed for this purpose.

Endometrial marker antibodies useful in treating disease (e.g. cancer) include those that are capable of initiating a potent immune response against the disease (e.g. tumor) and those that are capable of direct cytotoxicity. In this regard, endometrial marker antibodies may elicit cell lysis by either complement-mediated or antibody-dependent cell cytotoxicity (ADCC) mechanisms, both of which require an intact Fc portion of the immunoglobulin molecule for interaction with effector cell Fc receptor sites or complement proteins.

Endometrial marker antibodies that exert a direct biological effect on tumor growth may also be useful in the practice of the invention. Such antibodies may not require the complete immunoglobulin to exert the effect. Potential mechanisms by which such directly cytotoxic antibodies may act include inhibition of cell growth, modulation of cellular differentiation, modulation of tumor angiogenesis factor profiles, and the induction of apoptosis. The mechanism by which a particular antibody exerts an anti-tumor effect may be evaluated using any number of *in vitro* assays designed to determine ADCC, antibody-dependent macrophage-mediated cytotoxicity (ADMMC), complement-mediated cell lysis, and others known in the art.

The anti-tumor activity of a particular endometrial cancer marker antibody, or combination of endometrial cancer marker antibodies, may be evaluated *in vivo* using a suitable animal model. Xenogenic cancer models, where human cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice, may be employed.

The methods of the invention contemplate the administration of single endometrial marker antibodies as well as combinations, or "cocktails", of different individual antibodies such as those recognizing different epitopes of other markers. Such cocktails may have certain advantages inasmuch as they contain antibodies that bind to different epitopes of endometrial markers and/or exploit different effector mechanisms or combine directly cytotoxic antibodies with antibodies that rely on immune effector functionality. Such antibodies in combination may exhibit synergistic therapeutic effects. In addition, the administration of one or more endometrial marker specific antibodies

may be combined with other therapeutic agents, including but not limited to chemotherapeutic agents, androgen-blockers, and immune modulators (e.g., IL2, GM-CSF). The endometrial marker specific antibodies may be administered in their "naked" or unconjugated form, or may have therapeutic agents conjugated to them.

The endometrial marker specific antibodies used in the methods of the invention may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the antibodies retains the function of the antibody and is non-reactive with the subject's immune systems. Examples include any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16.sup.th Edition, A. Osal., Ed., 1980).

One or more endometrial marker specific antibody formulations may be administered via any route capable of delivering the antibodies to the a disease (e.g. tumor) site. Routes of administration include, but are not limited to, intravenous, intraperitoneal, intramuscular, intratumor, intradermal, and the like. Preferably, the route of administration is by intravenous injection. Antibody preparations may be lyophilized and stored as a sterile powder, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

Treatment will generally involve the repeated administration of the antibody preparation via an acceptable route of administration such as intravenous injection (IV), at an effective dose. Dosages will depend upon various factors generally appreciated by those of skill in the art, including the type of disease and the severity, grade, or stage of the disease, the binding affinity and half life of the antibodies used, the degree of endometrial marker expression in the patient, the extent of circulating endometrial markers, the desired steady-state antibody concentration level, frequency of treatment, and the influence of any chemotherapeutic agents used in combination with the treatment method of the invention. Daily doses may range from about 0.1 to 100 mg/kg. Doses in the range of 10-500 mg antibodies per week may be effective and well tolerated, although even higher weekly doses may be appropriate and/or well tolerated. A determining factor in defining the appropriate dose is the amount of a particular antibody

necessary to be therapeutically effective in a particular context. Repeated administrations may be required to achieve disease inhibition or regression. Direct administration of one or more endometrial marker antibodies is also possible and may have advantages in certain situations.

Patients may be evaluated for serum cancer markers in order to assist in the determination of the most effective dosing regimen and related factors. The endometrial cancer assay methods described herein, or similar assays, may be used for quantitating circulating endometrial marker levels in patients prior to treatment. Such assays may also be used for monitoring throughout therapy, and may be useful to gauge therapeutic success in combination with evaluating other parameters such as serum levels of endometrial markers.

The invention further provides vaccines formulated to contain one or more endometrial marker or fragment thereof.

In an embodiment, the invention provides a method of vaccinating an individual against one or more endometrial marker listed in Table 1 comprising the step of inoculating the individual with the marker or fragment thereof that lacks activity, wherein the inoculation elicits an immune response in the individual thereby vaccinating the individual against the marker.

The use in anti-cancer therapy of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity is well known and, for example, has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al., 1995, Int. J. Cancer 63: 231-237; Fong et al., 1997, J. Immunol. 159: 3113-3117). These and similar methods can be practiced by employing one or more endometrial markers, or fragment thereof, or endometrial polynucleotide markers and recombinant vectors capable of expressing and appropriately presenting endometrial marker immunogens.

By way of example, viral gene delivery systems may be used to deliver one or more endometrial polynucleotide markers. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, 1996, Curr. Opin. Immunol. 8: 658-663). Non-viral delivery systems may also be employed by using naked DNA encoding one or more

endometrial cancer marker or fragment thereof introduced into the patient (e.g., intramuscularly) to induce an anti-tumor response.

Various *ex vivo* strategies may also be employed. One approach involves the use of cells to present one or more endometrial marker to a patient's immune system. For example, autologous dendritic cells which express MHC class I and II, may be pulsed with one or more endometrial marker or peptides thereof that are capable of binding to MHC molecules, to thereby stimulate the patients' immune systems (See, for example, Tjoa et al., 1996, Prostate 28: 65-69; Murphy et al., 1996, Prostate 29: 371-380).

Anti-idiotypic endometrial marker specific antibodies can also be used in therapy as a vaccine for inducing an immune response to cells expressing one or more endometrial marker. The generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic endometrial cancer marker specific antibodies that mimic an epitope on one or more endometrial cancer markers (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J Clin Invest 96: 334-342; Herlyn et al., 1996, Cancer Immunol Immunother 43: 65-76). Such an antibody can be used in anti-idiotypic therapy as presently practiced with other anti-idiotypic antibodies directed against antigens associated with disease (e.g. tumor antigens).

Genetic immunization methods may be utilized to generate prophylactic or therapeutic humoral and cellular immune responses directed against cells expressing one or more endometrial cancer marker. One or more DNA molecules encoding endometrial markers, constructs comprising DNA encoding one or more endometrial markers/immunogens and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded endometrial markers/immunogens. The endometrial markers/immunogens may be expressed as cell surface proteins or be secreted. Expression of one or more endometrial markers results in the generation of prophylactic or therapeutic humoral and cellular immunity against the disease (e.g. cancer). Various prophylactic and therapeutic genetic immunization techniques known in the art may be used.

The invention further provides methods for inhibiting cellular activity (e.g., cell proliferation, activation, or propagation) of a cell expressing one or more endometrial marker. This method comprises reacting immunoconjugates of the invention (e.g., a

heterogeneous or homogenous mixture) with the cell so that endometrial markers form complexes with the immunoconjugates. A subject with a neoplastic or preneoplastic condition can be treated when the inhibition of cellular activity results in cell death.

In another aspect, the invention provides methods for selectively inhibiting a cell expressing one or more endometrial marker by reacting any one or a combination of the immunoconjugates of the invention with the cell in an amount sufficient to inhibit the cell. Amounts include those that are sufficient to kill the cell or sufficient to inhibit cell growth or proliferation.

Vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used to deliver polynucleotides encoding endometrial cancer markers to a targeted organ, tissue, or cell population. Methods well known to those skilled in the art may be used to construct recombinant vectors that will express antisense polynucleotides for endometrial markers. (See, for example, the techniques described in Sambrook et al (supra) and Ausubel et al (supra)).

Methods for introducing vectors into cells or tissues include those methods discussed herein and which are suitable for *in* vivo, *in vitro* and ex *vivo* therapy. For *ex vivo* therapy, vectors may be introduced into stem cells obtained from a patient and clonally propagated for autologous transplant into the same patient (See U.S. Pat. Nos. 5,399,493 and 5,437,994). Delivery by transfection and by liposome are well known in the art.

Genes encoding endometrial markers can be turned off by transfecting a cell or tissue with vectors that express high levels of a desired endometrial marker-encoding fragment. Such constructs can inundate cells with untranslatable sense or antisense sequences. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies are disabled by endogenous nucleases.

Modifications of gene expression can be obtained by designing antisense molecules, DNA, RNA or PNA, to the regulatory regions of a gene encoding an endometrial marker, i.e., the promoters, enhancers, and introns. Preferably, oligonucleotides are derived from the transcription initiation site, e.g. between -10 and +10 regions of the leader sequence. The antisense molecules may also be designed so that they block translation of mRNA by preventing the transcript from binding to ribosomes. Inhibition may also be achieved using "triple helix" base-pairing

methodology. Triple helix pairing compromises the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Therapeutic advances using triplex DNA were reviewed by Gee J E et al (In: Huber B E and B I Carr (1994) Molecular and Immunologic Approaches, Futura Publishing Co, Mt Kisco N.Y.).

Ribozymes are enzymatic RNA molecules that catalyze the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. The invention therefore contemplates engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding an endometrial marker.

Specific ribozyme cleavage sites within any potential RNA target may initially be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once the sites are identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be determined by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

One or more endometrial markers and polynucleotides encoding the markers, and fragments thereof, may be used in the treatment of an endometrial disease (e.g. endometrial cancer) in a subject. The markers or polynucleotides may be formulated into compositions for administration to subjects suffering from an endometrial disease. Therefore, the present invention also relates to a composition comprising one or more endometrial markers or polynucleotides encoding the markers, or a fragment thereof, and a pharmaceutically acceptable carrier, excipient or diluent. A method for treating or preventing an endometrial disease in a subject is also provided comprising administering to a patient in need thereof, one or more endometrial markers or polynucleotides encoding the markers, or a composition of the invention.

The invention further provides a method of inhibiting an endometrial disease (e.g. endometrial cancer) in a patient comprising:

(a) obtaining a sample comprising diseased cells from the patient;

(b) separately maintaining aliquots of the sample in the presence of a plurality of test agents;

(c) comparing levels of one or more endometrial markers, and/or polynucleotides encoding one or more endometrial markers in each aliquot;

(d) administering to the patient at least one of the test agents which alters the levels of the endometrial markers, and/or polynucleotides encoding one or more endometrial markers in the aliquot containing that test agent, relative to the other test agents.

Endometrial markers in uterine biopsy tissue or fluid and sera may vary between known fertile and infertile women during the window of implantation, deviate in women undergoing ovarian hyperstimulation/ovulation induction, and correlate with successful initiation of pregnancy. Therefore, endometrial markers of the invention may serve as minimally or noninvasive markers of uterine receptivity for implantation.

The present invention further provides a method of determining uterine endometrial receptivity by first obtaining a serum, uterine fluid or endometrial biopsy sample from a patient and detecting the presence of an endometrial marker associated with a certain endometrium phase, wherein the presence or absence of an endometrial marker as compared to controls indicates uterine receptivity. In an embodiment, the endometrium phase is the secretory phase. Where necessary for the evaluation, repetitive samples may be collected throughout the menstrual cycle. Non-receptive controls are both women who are in the non-fertile stage of the menstrual cycle and women with known uterine dysfunction where an endometrial marker is not present or present on the endometrium throughout the menstrual cycle or certain endometrium phases.

The present invention further provides a method of monitoring the effects of ovarian hyperstimulation and/or ovulation induction protocols on uterine receptivity either for individual women receiving the treatment or for the evaluation of new protocols. In an embodiment, the method comprises: (a) obtaining a serum, uterine or fluid or endometrial biopsy sample from a patient receiving the treatments; and (b) detecting the presence of an endometrial marker of the invention present in the endometrium at the time of fertilization, early embryogenesis, and implantation; wherein presence or absence of an endometrial marker indicates receptivity. A disruption of the

normal cyclic presence of an endometrial marker indicates that the treatment may adversely affect uterine receptivity. This disruption may include non-cyclic presence of an endometrial marker or an aberrant presence of an endometrial marker as compared to controls.

In an aspect the invention provides a method of determining a probability of successful implantation with an ovarian stimulation *in vitro* fertilization and embryo transfer procedure, comprising:

(a) determining a level of an endometrial marker identified in accordance with a method of the invention in a sample obtained from a patient who has undergone an ovarian stimulation *in vitro* fertilization and embryo transfer procedure; and

(b) determining a probability of successful implantation based on the patient's determined endometrial marker level;

wherein a significantly different endometrial marker level relative to a standard level is associated with a decreased or increased probability of successful implantation.

The present invention further provides a method of contraception by interrupting the cyclic presence of an endometrial marker. The interruption can be to reduce or eliminate a marker present during the uterine receptivity window for implantation of the menstrual cycle and to thereby alter the cyclic presence/pattern of a marker. The interruption can utilize an antagonist of a marker. The term antagonist or antagonizing is used in its broadest sense. Antagonism can include any mechanism or treatment that results in inhibition, inactivation, blocking or reduction or alteration of cyclic presence of an endometrial marker.

An active therapeutic substance described herein may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active substance may be coated in a material to protect the substance from the action of enzymes, acids and other natural conditions that may inactivate the substance. Solutions of an active compound as a free base or pharmaceutically acceptable salt can be prepared in an appropriate solvent with a suitable surfactant. Dispersions may be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, or in oils.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the active substances in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

The compositions are indicated as therapeutic agents either alone or in conjunction with other therapeutic agents or other forms of treatment. The compositions of the invention may be administered concurrently, separately, or sequentially with other therapeutic agents or therapies.

The therapeutic activity of compositions and agents/compounds identified using a method of the invention and may be evaluated *in vivo* using a suitable animal model.

The following non-limiting examples are illustrative of the present invention:

Example 1

Isotope-coded affinity tag (ICAT) analysis with a cleavable tag was used to examine differentially expressed proteins in proliferative and secretory endometria. Sample complexity of the tissue homogenates was reduced by strong cation exchange (SCX) fractionation and subsequent affinity cleanup. Analysis of ten SCX fractions in triplicate by nanobore liquid chromatography – tandem mass spectrometry (nanoLC-MS/MS) resulted in the identification of approximately 400 labeled proteins and the discovery of potential biomarkers for the secretory phase of the endometrial cycle. This study demonstrated the feasibility of using this approach for identifying markers at a proteomics level for different stages of the endometrial cycle.

Tissue Samples

Endometrium tissue was retrieved from an in-house dedicated, research endometrial tissue bank. All tissues were snap frozen in liquid nitrogen within 15-20 minutes of devitalization at the time of hysterectomy, and were obtained with patient consent. In each case, the endometrium was classified as proliferative or secretory by a

pathologist (TJC). The histological classification was verified by examination of a histopathologic section from the frozen research tissue. Tissue was taken for proteomic analysis from the mirror-face of the residual block. After addition of 1 ml Hanks' Balanced Salt Solution containing protease inhibitors (leupeptin, aprotinin, pepstatin at 1 µg/mL), the tissue was mechanically homogenized at 30,000 rpms using a Polytron PT 1300D handheld homogenizer (Brinkmann, Westbury, USA). The samples were stored in aliquots at -80°C and/or submitted for protein profiling. These whole tissue homogenates contain endometrial epithelium, supportive stroma and vessels, as well as any secretions. Tissue samples from six different individuals were selected for the study. Three of these tissues were classified as proliferative endometria (PRO1, PRO2 and PRO3) and the other three as secretory endometria (SEC1, SEC2, SEC3).

Chemicals

Acetonitrile, formic acid, potassium chloride, monobasic potassium phosphate, leupeptin, aprotinin, pepstatin and Hanks' Balanced Salt Solution were obtained from Sigma-Aldrich (Oakville, Canada). All reagents and buffers for the cleavable ICAT sample preparation procedure were from Applied Biosystems (Foster City, USA).

ICAT Sample Preparation Procedure

After removal of cell debris by centrifugation, the total protein content for each of the six clarified homogenates was measured using a commercially available Bradford protein assay reagent (Bio-Rad, Hercules, USA). ICAT sample preparation procedure was carried out according to the cleavable ICAT protocol (Applied Biosystems, Foster City, USA) and is illustrated in Figure 1. Following denaturing and reducing steps, 100 µg total protein of each of the samples was labeled with either the light ICAT reagent (proliferative samples) or the heavy reagent (secretory samples). The labeled PRO1 and SEC1 samples were combined to form ICAT Sample A, PRO2 and SEC2 form ICAT Sample B, and lastly PRO3 and SEC3 form ICAT Sample C. Mixing of the labeled proliferative and secretory samples in pairs in this manner ensures that any protein or peptide losses during subsequent processing steps is the same for both samples in a pair. Since the peptides are differentially labeled, they can be traced to specific samples in the pair. Any difference detected in the levels of individual peptides can then be ascribed solely to initial differences in expression level. Chromatographic separation using a strong cation exchange (SCX) column was performed after trypsin digestion to

fractionate the ICAT samples. Selected SCX fractions were purified by affinity chromatography according to the ICAT protocol, and subsequently analyzed by nanobore liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS).

Instrumentation for SCX fractionation

SCX fractionation was performed on an HP1050 LC system (Agilent, Palo Alto, USA) using a 1.5 ml injection loop and a SF-2120 Super Fraction Collector (Advantec MFS, Dublin, USA).

LC/MS/MS Instrumentation

The LC system from LC Packings (Amsterdam, The Netherlands) consisted of a Famos autosampler and Ultimate Nano LC system. It was interfaced to an API QSTAR Pulsar QqTOF mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, USA) using a Protana NanoES ion source (Protana Engineering A/S, Odense, Denmark). PicoTip's SilicaTip emitters with a 10 µm tip i.d. (New Objective, Woburn, USA) were used as spray capillaries. All data were acquired using Analyst QS SP5 with Bioanalyst Extension 1.1 and analyzed with ProICAT SP2 software (Applied Biosystems/MDS Sciex, Foster City, USA).

LC Conditions

Strong cation exchange chromatography (SCX) was performed using a PolyLC Polysulfoethyl A column (The Nest Group, Southborough, USA) equipped with a guard column of the same material with the following dimensions: 5-µm particle size, 300-Å bead, 2.1-mm i.d., 10-mm length (guard column) and 100-mm length (analytical column). Eluent A of the mobile phase consisted of a 10 mM KH_2PO_4 solution in 25% acetonitrile and 75% deionized water (pH = 3.0). Eluent B consisted of a 10 mM KH_2PO_4 and 350 mM KCl solution in 25% acetonitrile and 75% deionized water (pH = 3.0). In each case, 1.5 ml of the total 2.4 ml sample (after acidifying with 2 ml Eluent A) was injected. Fractions were collected every 2 mins at a flow rate of 0.2 ml/min. using a binary gradient with the following profile:

t [min]	0.01	2	58	60
c(Eluent B) [%]	0	0	100	stop

All reversed-phase separations were performed using PepMap C18 nano capillary columns (LC Packings, Amsterdam, The Netherlands) with the following dimensions: 3-µm particle size, 100-Å bead, 75-µm i.d. and 150-mm length. Eluent A of the mobile phase consisted of 950 ml deionized water, 50 ml acetonitrile and 1 ml formic acid (pH ≈ 3). Eluent B consisted of 50 ml deionized water, 950 ml acetonitrile and 1 ml formic acid. A binary gradient at a flow rate of approximately 200 nl/min with the following profile was used:

t [min]	0.01	5	125	135	157	160	163	190
c(Eluent B) [%]	5	5	30	60	80	80	5	Stop

Injections of 1 µl of sample were performed in full loop mode.

MS Conditions

The source conditions were a curtain-gas setting of 20 and an ionspray voltage (in the range of 2000 - 3800 V) that was optimized daily. All data were obtained in the positive-ion detection mode. In the Q0 region, the instrument parameters were a declustering potential (DP) of 65 V and a focusing potential (FP) of 265 V. Nitrogen was used as the collision gas at a setting of CAD5 for both TOF-MS and MS/MS scans.

All LC-MS/MS data were acquired in information-dependent acquisition (IDA) mode. A TOF-MS survey scan with a mass range of m/z = 400 - 1500 and 1 s scan time was followed by two product ion scans with a mass range of m/z = 70 – 2000 and 2 s scan time. The collision energy (CE) was automatically controlled by the IDA CE Parameters script. The switching criteria were set to ions greater than m/z = 400 and smaller than m/z = 1500 with a charge state of 2 to 5 and an intensity of ≥ 10 counts/s. Former target ions were excluded for 60 s and peaks within a 4 Th window were ignored. In addition, the IDA Extensions II script was set to 2 repetitions before dynamic exclusion and to select a precursor ion nearest to a threshold of 15 count/s every 4 cycles.

RESULTS AND DISCUSSION

Figure 2 shows examples of the histologic appearance of proliferative (Figure 2a) and secretory (Figûre 2b) endometrium. In both endometria the stratum basalis is characterized by a denser stroma than the physiologic responsive stratum functionalis above. Across the top of the stratum functionalis is the surface epithelium, which lines the endometrial cavity. The proliferative endometrium (PRO 2) shows small, coiled

glands with lining columnar epithelium reaching to the surface. In contrast, the secretory endometrium (SEC2) is thicker, and contains more tortuous glands with intra-luminal secretions. The endometrium of both types of physiologic phases has abundant supportive stroma and vessels among the epithelial glands. In this study homogenates from the mirror faces of these tissues were used for quantitative analysis. After performing the procedure described above, SCX fractions 11 - 20 (of the 30 fractions) from each of the samples were chosen for further processing. This choice was based on the UV trace generated during fractionation. The 10 chosen fractions from each sample were affinity-purified, cleaved as per the ICAT protocol and analyzed using nanoLC-MS/MS. Figure 3 is an example of a nano LC-MS total ion chromatogram (TIC) from one of the ICAT Sample A SCX fractions. As the samples were run in IDA mode (See above), each such TIC resulted in hundreds of MS/MS spectra. A ProICAT confidence value of 75 was adopted after trial-and-error for reliable protein detection and identification for this initial investigation. This has resulted in identification of approximately 400 distinct proteins. A preliminary classification of these proteins based on function, is seen in Figure 4. As expected the majority of proteins fall under one of the metabolic, housekeeping or structural categories. The proteins classified under "other" are proteins like antibodies, which could not be included with the previously mentioned categories. There were also a significant number of proteins for which function could not be assigned or were identified from cDNA matches and are therefore classified as hypothetical. This last category often contains the most interesting cases for biologists. Adopting a less stringent confidence value resulted in apparent identification of many more proteins; the reliability of such identifications, however, was judged much poorer after manual inspection. In addition, manual inspection of the spectra was also found necessary to confirm automated quantifications in ProICAT.

The results show that expression levels of the majority of proteins identified were not consistently different between the two phases of the endometrial cycle. This similarity in expression levels of proteins is not surprising since most of the abundant proteins detected tend to be housekeeping or structural in nature. In addition, similar or identical, housekeeping or structural proteins such as actin and tubulin are expressed in many tissue types. This is significant since whole tissue homogenates consist not only of epithelial cells, but also supportive stromal cells and interstitium, blood, vessels and any

glandular secretions. These sources provide a major contribution to the protein profile of the overall tissue. These high abundance proteins from these adjacent cellular types may mask differential protein expression by a cellular component of the endometrium. Thirdly the differential expression of low abundance proteins in any one cellular type within proliferative and secretory endometrium may not have been detectable by the methodology used in this study.

Despite these limitations, some instances of differential protein expression was noted. One such example is given in Figure 5, which shows very significant enhancement of expression of a protein, identified as glutamate receptor subunit zeta 1 precursor [SEQ ID NO. 21], in all three secretory samples. The triply charged series of peaks, starting at 581.6 Th, was MS/MS-analyzed and identified as the heavy-labeled version of the tryptic peptide LLTLALLFSCSVAR [SEQ ID NO. 23], which maps to the N-terminal region of the protein. The absence of the light-labeled analogue of this protein, which would have manifested as a series of peaks starting at 578.6 Th, suggests a significantly lower level of the same protein in the proliferative samples. This is the first evidence of this protein being a marker for the endometrial secretory phase.

A second example is shown in Figure 6 for the protein, macrophage migration inhibitory factor (MIF). Again, the protein's expression is enhanced in the secretory endometrium (1.71 ± 0.38 times) versus the proliferative endometrium. This relative quantification is based on the three sets of samples and is calculated from the ratios of the total area of the ion peaks within the heavy-labeled series to that of the corresponding light-labeled series. Previous studies have demonstrated that MIF is expressed by the human endometrium throughout the menstrual cycle and that this expression is predominantly in the glandular epithelial cells [9]. It was found that MIF localized throughout the glandular epithelial cytoplasm in the proliferative phase, but that this distribution changed during the secretory phase, when it localized to the apical portion of the glandular epithelial cells, and was also detected in glandular secretions. Macrophages are common in female reproductive tissues. In the endometrium, they play an important role in defense. Macrophage degradation of cellular debris and foreign material may play an important role in endometrial shedding and repair. Quantification of MIF levels using ELISA assays suggested a slight increase in the mean concentration in the secretory phase (from approximately 15 to 18 ng / mg of protein based on a total of 25

samples), although the increase was not statistically significant [9]. By contrast, ICAT analysis of the six-sample set shows a significant enhancement (1.71 ± 0.38 times) of MIF expression in the secretory endometrium. The results of this study serve to illustrate the power of the ICAT method for detecting and quantifying gross as well as subtle differences in expression levels over traditional quantitative methods relied on thus far.

CONCLUSIONS

The ICAT technology employing the new, cleavable ICAT reagent is a powerful tool that can be used for discovering differentially expressed proteins, which could potentially be significant biomarkers of different histological cell states. The results demonstrate that for the human endometrium the expression levels of the majority of proteins do not vary significantly between the proliferative and the secretory phases. Two examples of differentially expressed proteins have been discussed in this study. Further investigation of the remainder of the SCX fractions from each of the three pairs of samples can be expected to yield more markers some of which might have implications for fertility/ infertility.

Example 2

The ICAT analysis as described in Example 1 was used to examine differentially expressed proteins in cancer and normal endometrial tissue. The results of the anaylsis are illustrated in Figure 11 which shows mass spectral windows from the ICAT experiments for three pairs of endometrial cancer/ normal samples, demonstrating the over expression of calgizzarin in the cancer samples.

Example 3

Material and Methods:

i) Tissue preparation and histologic classification

Endometrium and endometrial cancer tissues were retrieved from a dedicated, research in-house endometrial tissue bank. The consenting and tissue banking procedures for this tissue bank were approved by the relevant institutions. All tissues had been snap frozen in liquid nitrogen within 15-20 minutes of devitalization at the time of hysterectomy, and were obtained with patient consent. In each case, the endometrium was classified as non-malignant, or malignant by a pathologist. (26). Non-malignant endometrial cases included both normal physiologic states (atrophic, proliferative, secretory, menstrual) and pathologic states (benign endometrial polyp, disordered

proliferative). Malignant endometrial cases included endometrioid, mucinous, and serous adenocarcinomas and malignant mixed Mullerian tumors (carcinosarcomas). This classification was performed using the routine surgical pathology sections. Study cases included only benign or malignant cases; cases of endometrial hyperplasia, some of which could be considered to represent an intermediate phenotype, were not included in this study. The histologic classification was verified by examination of a histopathologic section from the frozen research tissue. Tissue was taken for proteomic analysis from the mirror-face of the residual block.

Tissue was thawed in Hanks' balanced salt solution (HBSS, Sigma) containing protease inhibitors (leupeptine, aprotinin, pepstatin in 1 µg/mL) and followed by mechanical homogenation. The specimens were then stored in aliquots at -80°C and/or submitted for protein profiling. These whole tissue homogenates contain both endometrial epithelium or carcinoma, supportive stroma and vessels, and any secretions.

Immunohistochemical staining of selected malignant endometrial tissue was done using a polyclonal (rabbit) antibody against the putative tumor marker available from Calbiochem (San Diego, CA). Sections were cut from the paraffin embedded tissue, antibody applied in a 1:2000 dilution in Universal Strepavidin System, and immunohistochemical completed using a diaminobenzidine (DAB) chromogen.

ii) Protein profiling

Tissue lysate was fractionated to reduce the sample complexity before protein profiling. An identical quantity of proteins was used for all samples within a method; HBSS was used to compensate the initial volume to ensure equal volumes for all samples. For C18 Zip-tip (Millipore) fractionation, 2 µg of proteins from endometrial tissue homogenate in the presence of 0.3% trifluroacetic acid (TFA) were loaded. After washing with water containing 0.3% TFA, 1 µL of 60% acetonitrile with 0.3% TFA were used to elute proteins from C18 directly onto a MALDI target containing pre-dried 1 µL 10 mg/mL sinapinic acid in 60% acetonitrile. The dried protein spots were analyzed by a MALDI-TOF (Voyager DE-STR, Applied Biosystems) mass spectrometer.

For protein profiling using SELDI-TOF MS, 1 µg proteins from endometrial tissue homogenate were incubated with WCX2, SAX2, IMAC, H50 surfaces according to the manufacturer's instructions. In brief, samples were diluted to 55 µL with the corresponding binding buffer, spotted onto the appropriate ProteinChip surface, and

incubated in a sealed BioProcessor for one hour at room temperature. The ProteinChip surface was washed twice with the appropriate buffer for five-minutes, briefly rinsed with water and air-dried. Two times 0.5 μL of 50% saturated sinapinic acid in 50% acetonitrile was applied on the samples to form crystals. The ProteinChips were analyzed using a linear TOF analyzer, PBSIIc (Protein Biology System IIc, Ciphergen), or a quadrupole/TOF hybrid tandem mass spectrometer, QSTAR XL (Applied Biosystems/MDS Sciex).

iii) Protein purification and identification

A normal and an EmCa sample were subject to chromatographic separation in parallel to yield partially purified protein for identification. 500 μg proteins from the whole tissue homogenate were fractionated using size exclusion (BioSep 2000, Phenomenex) at 1mL/min flow with phosphate buffer (pH7.9) and 0.05% (w/v) sodium azide. One-millilitre fractions were collected; the eluates were then concentrated to 50 μL with a silicon carbide-based spincolumn, (ProteoSpin, MDS Sciex). Five microlitres (10%) of concentrate was desalted by C18 zip-tip and analyzed with MALDI-TOF MS to locate the fractions containing the protein marker of interest (10,843 Da) in the EmCa sample. The fractions with the enriched 10,843 Da protein were diluted to 100 μL with freshly prepared dithiothreitol (DTT) (5 mM final) in 150 mM Tris pH 8.5 buffer, and incubated at 60 Cfor one hour. Ten microlitres (10%) of the reaction mixture was desalted by C18 zip-tip and analyzed with MALDI-TOF MS to assess the effect of DTT on the protein of interest (see Result for detail). The remaining 90 μL was precipitated by acetone (80%(v/v) final), resuspended in SDS sample buffer, and the proteins were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Protein molecular weight markers (New England Biolabs) and cytochrome C (C-2506 Sigma) were included to guide the excision of gel portions containing the protein of interest. Intact proteins were extracted from the gel by 50 μL extraction solution (formic acid/acetonitrile/isopropanol/water in a ratio of 50/25/15/10) at room temperature for four hours. The extracts were completely dried by SpeedVac and resuspended in 40 μL 100 mM ammonium bicarbonate. Half of the resuspended proteins was desalted by C18 zip-tip and analyzed with MALDI-TOF, the other half was digested in solution with 100 ng trypsin (Promega). The resulted tryptic peptides were analyzed by MALDI-QqTOF

MS. The identity of the 10,843Da protein, chaperonin 10, was determined using amino acid sequence-tag analysis (Mascot, Matrix Science).

Identification of chaperonin 10 was verified by western blot, 12 μg of proteins was resolved by 8-16% gradient SDS-PAGE and electrophoretically transferred to nitrocellulose membrane (MSI). Membranes were incubated for two hours with 1:1000 dilution of anti-chaperonin 10 antiserum (Stressgen) or ERK1 antibodies (Santa Cruz) diluted in 5% milk with 0.2% NP-40 and one hour with secondary antibodies. Chaperonin 10 and ERK1 were detected by chemiluminescence reagent (NEN) and X-ray film according to the manufacturer's instructions.

Results:

A total of 44 malignant and non-malignant endometrial tissue samples were submitted for proteomic analysis. Twenty-three of these cases were non-malignant, and the remaining 21 cases were malignant. The exact histopathologic diagnoses are shown in Tables 2 and 3.

Protein profiling

Both C18 zip-tip purification and retentive separation on ProteinChip WCX2 were effective in generating protein profiles that permit differentiation between normal and tumorous epithelium samples. Distinguishing features include both appearances and disappearances of proteins. One protein at approximately 10,840 $\pm$ 10 Da was present in all EmCa samples and absent or greatly diminished in all normal samples tested. Figure 7 shows the mass spectra obtained on the ProteinChip WCX2 using (a) the linear TOF mass spectrometer, PBSIIc, and (b) the QqTOF mass spectrometer, QSTAR XL. The superior mass accuracy and resolution of the latter afforded determination of the molecular weight of the marker protein as 10,843 Da.

Marker protein purification and identification

As detailed above and outlined in Figure 8 size-exclusion LC was employed to fractionate the tissue homogeneates. The target 10,843 Da protein was found to elute in one of the early fractions from size exclusion column which suggests that this protein is a part of a large protein complex (Figure 8a). After concentration on the spin column, the proteins were treated with DTT to break the tertiary structure and reduce any disulfide bonds. The molecular weight of the target protein was verified to remain as 10,843 Da after DTT treatment and before SDS-PAGE (Figure 8b). This result suggests that the

10,843 Da target protein contains no intra- or inter-polypeptide disulfide bonds. The remaining protein concentrates were further separated by SDS-PAGE, and the gel was stained with colloidal Coomassie Blue (Figure 8c). There were, however, no visible bands at the region around 10-11 kDa, probably a consequence of low protein concentration and relatively low sensitivity of the Coomassie stain. The gel portions covering approximate 7,000-16,000 Da were excised as guided by the molecular weight markers (Figure 8c). After protein extraction, digestion with trypsin and MALDI analysis, six "unique" tryptic peptides were detected in the EmCa sample vs. the control (Figure 8d). All six peptides were sequenced by MALDI-QqTOF MS; three were traced to keratin and three to chaperonin 10 (Figure 8d and 8e). The average molecular weight of chaperonin 10 was calculated to be 10842.5 Da after considering two putative posttranslational modifications, the removal of the N-terminal methionine and acetylation of the alanine residue. This is consistent with the measurement of the target protein and the reported molecular weight of chaperonin 10 purified from human platelet (18).

The differential expression of chaperonin 10 among cancerous and normal tissue was re-tested by western blot analysis. The signal of chaperonin 10 is higher in all EmCa specimens that also display a relative high 10,843 Da peak in their corresponding protein profiles (Figure 9).

Tables 2 and 3 summarizes the results in identifying chaperonin 10 by MS and western blot analyses in non-malignant and malignant endometrial tissue respectively. The results for both MS and western blotting have been reported using a semi-quantitative system ranging from absent (0) to 5+ (for high intensity). These two independent methodologies demonstrate consistency in the detection of chaperonin 10.

Moderately strong immunohistochemical staining for chaperonin 10 was noted in the cytoplasm of an endometrioid adenocarcinoma, as compared to adjacent stroma and benign endometrial gland. (Figure 10). This result demonstrates the association of chaperonin 10 with malignant endometrial tissues, but much less with normal endometrial epithelium or supportive stroma and vessels, which were also present in the whole tissue homogeneates.

Discussion:

Genomic studies of endometrial carcinoma have revealed that there are two, and possibly three, types of endometrial carcinoma that are each characterized by multistep pathogenetic pathways characterized by different molecular profiles (28). A wide variety of genetic and enzymatic markers characterize the initiation, promotion and progression toward each type of endometrial carcinoma. Large-scale messenger RNA expression analysis of the endometrioid type of endometrial carcinoma has identified 50 genes that are capable of discriminating normal from malignant endometrial tissues.(29) Many genes that are constitutively expressed in normal endometrium show either diminished or increased expression in endometrial carcinomas. In addition, there is aberrant expression of the one hundred hormonally regulated genes that are variably expressed in normal endometrial tissues, although finally endometrial carcinoma resembles proliferative endometrium more than secretory endometrium (29).

In contrast to the genomic studies of the endometrium, no studies of the endometrial proteome are available, even though proteome analysis may offer information about protein expression, functions, and modifications which might not be fully reflected by gene expression analysis. Thus, protein expression profiling of the endometrium offers a new opportunity to identify and classify endometrial phenotypes, including carcinoma. Among the available methodologies for protein profiling, SELDI-TOF based method has the advantage of requiring only miniscule samples and high throughput capability, although the determination of any protein identity requires much more work (30).

This proteomic study of endometrium used lysates of whole tissue homogenates from both control endometrial tissues and endometrial carcinomas. Such tissues include not only the epithelial cells of interest, but also supportive stroma (including both endometrial stromal cells or fibroblasts and extracellular matrix), blood vessels (including smooth muscle cells and endothelium), any secretions, and possibly small amounts of adjacent myometrium. While the use of such whole tissue homogenates is technically straight forward, it does have inherent limitations. The heterogeneous nature of the constituent tissue will lead to a similarly heterogeneous protein profiling (17). The resultant proteomic analysis reflects not only the cells of interest, but also the presence of contaminating cells (15, 17, 31). Successful proteomic analysis of some types of tumors (e.g. hepatoma) may still be productive even with the limitation of whole tissue

homogenization, since there is an abundance of tumor cells and minimal associated contaminating stroma (32).

In some tumor types, the inherent limitation of whole tissue homogenates must be surmounted by utilizing cell purification techniques. In the endometrium LCM may be optimal in achieving cellular purification for proteomic analysis since there is a relative abundance of stroma in endometrial carcinoma tissues (32). Studies using laser capture microdissection (LCM) of melanoma have clearly revealed a different protein profile for melanoma than that of the surrounding epithelium, and thus supports the use of LCM in proteomic profiling studies (21, 33-35).

Despite the limitations of using whole tissue homogenates for protein expression profiling, this preliminary study of endometrial carcinoma has shown that proteomic analysis of endometrial carcinoma can detect differences from that of normal endometrium. Furthermore, a specific protein (Chaperonin 10) was strongly associated with endometrial carcinoma cases. This potential marker might be clinically useful on tissue aspirates since its differential expression pattern can be detected without the LCM procedure.

Chaperonin 10 was not identified exclusively in malignant endometrial tissues; low levels were detected in non-malignant endometrial tissues by both mass spectrometry and western blotting techniques (Table 2). Furthermore, there is no apparent association between any particular histopathologic classification and the detection of these low levels of chaperonin 10. In contrast, high levels of chaperonin 10 were detected in 17 of 22 malignant endometrial tissues by either mass spectrometry and/or western blotting techniques (Table 3). The apparent absence of chaperonin 10 in the remaining five of the 22 malignant cases may be due to either true absence or technical factors in pre-analytic processing or proteomic analysis. In two of these five cases, re-examination of the corresponding mirror image histologic section revealed minimal tumor in one case (case 28), or abundant necrosis of tumor (case 44). Furthermore, specific protein peaks of interest may be obscured in less-than-optimal mass spectromectric analysis or by adjacent protein peaks. Clearly, additional studies will be needed to confirm both sensitivity and specificity of chaperonin 10 as a marker of endometrial malignancy.

Single new biomarkers, such as Chaperonin 10, could have significant overlap with non-neoplastic states which may only be detectable after detailed study of a larger number of cases (23). Therefore, it is probable that the identification and combined use of a number of biomarkers will provide more optimal identification of malignant diseased states. Proteomic studies of pure endometrial cancer cellular lysates should better define the exact expression protein profile of this disease, and new biomarkers, as well. Such protein profiling does hold promise as a tool to identify new biomarkers of endometrial disease that could serve as new diagnostic or screening techniques in the future.

Chaperonin 10 (Cpn 10) is a heat shock protein (HSP) that functions intra-cellularly as a molecular chaperone for nascent proteins (36, 37). HSP's are ubiquitous intra-cellular proteins that ensure homeostasis of metabolism (37). Aberrations of HSP function, including chaperonin 10, have been described in a variety of pathologic conditions, including neoplasia (37, 38). Furthermore, HSP's are differentially expressed in a variety of neoplasms. For example, immunohistochemical studies of both primary and secondary brain tumours have shown production of other HSP's (39), and that the expression of some HSP's may depend upon proliferating potential. Furthermore, the modulation of HSP expression profile has been shown to reflect the stage of prostatic carcinoma (37).

Immunohistochemical studies have identified HSP's within the nuclei and cytoplasm of epithelium, stroma, endothelium, and lymphocytes of the endometrium, with certain types of HSP's showing preferential localization to certain cell types (40). Whereas the expression of some HSP's occurs independent of the stage of the endometrial cycle (e.g. HSP90), the expression of other HSP's is cycle dependent. The expression of chaperonin 10 can be determined among proliferative, secretory, and menstrual endometria using proteomic, western blotting, and immunohistochemical methods. It is known that the amount of HSP27 and 60 is increased during late proliferative and early secretory phases, and subsequently reduced during mid- and late secretory and menstrual phases (40). Studies of both decidualized endometrium (decidua) and placenta have shown that there are striking differences in the cellular localization of HSP's during normal human gestation.

There have been no studies regarding the role of HSP's or Cpn10 in endometrial carcinoma. The eutopic endometrial glands from women with endometriosis and adenomyosis shows significantly increased expression of HSP's as compared to endometria from control women – regardless of the menstrual phase (41). The abnormal expression of HSP's may play a role in the pathophysiology of both endometriosis and adenomyosis.

Early pregnancy factor (EPF) is an extra-cellular homologue of Cpn 10 that appears within 24 hours of fertilization and persists throughout the first half of gestation (36). It is necessary for embryonic development (42-44) and is immunosuppressive (45). EPF is also detectable in animal models of liver regeneration and in the development of cancer (38, 46, 47). An association between cellular growth and the appearance of extracellular EPF has been shown (38, 46, 47). These findings suggest a role for EPF in neoplastic growth and that the detection of Cpn 10 in the serum of endometrial carcinoma has diagnostic potential.

In conclusion, this protein profiling study of non-malignant and malignant endometrial tissues has identified chaperonin 10 as a potential tumor marker for endometrial malignancies.

Table 1

Protein EmCa	*Gene name*	*Swiss-Prot Accession No.*	*Expression in*
Chaperonin 10	HSPE1	Q04984 [SEQ ID 1] NM_002157 and HSU07550 [SEQ ID 2]	Up
Calgranulin A	S100A8	P05109 [SEQ ID 3] NM_002964 and BT00378 [SEQ ID 4]	Up
Calgranulin B	S100A9	P06702 [SEQ ID 5] X06233 [SEQ ID 6]	Up
Polymeric-immunoglobulin Receptor [precursor]	PIGR	P01833 or Q81ZY7 [SEQ ID 7] NM_002644 [SEQ ID 8]	Up
Phosphatidylethanolamine-binding protein (PEBP)	PBP	P30086 [SEQ ID 9] BC031102 [SEQ ID 10]	Up
Acidic leucine-rich nuclear phosphoprotein 32 family member A	ANP32A	P39687 [SEQ ID 11] X75090 [SEQ ID 12]	Up
Heat shock 70 kDa protein 6	HSPA6	P17066 [SEQ ID 13] NM-002155 [SEQ ID 14]	Up
Macrophage migration Inhibitory factor (MIF)	MIF	P14174 [SEQ ID 15] NM_002415 [SEQ ID 16]	Up
Calgizzarin (S100C protein)	S100A11	P31949 [SEQ ID 17] NM_005620 [SEQ ID 18]	Up
Triosephosphate isomerase	TPI1	P00938 [SEQ ID 19] NM_000365 [SEQ ID 20]	Up

Table 2

The identification of Chaperonin 10 by mass spectrometry and Western blotting techniques in non-malignant endometrial tissue homogeneates.

Case	Histopathologic Classification	MS results (relative intensity)	Western Blotting Results (relative intensity)
1	Proliferative	+	o
2	Proliferative	+	o
3	Secretory	+	o
4	Atrophic	+	+
5	Benign polyp	o	o
6	Atrophic	+	+
7	Secretory	o	o
8	Disordered proliferative endometrium	o	+
9	Secretory	o	+
10	Disordered proliferative	o	+
11	Secretory	o	+
12	Secretory	+	+
13	Secretory	o	+
14	Secretory	+	o
15	Atrophic	o	o
16	Secretory	+	+
17	Menstrual	+	+
18	Secretory	+	+
19	Proliferative	+	o
20	Proliferative	+	+
21	Menstrual	o	o
22	Atrophic	o	o
23	Atrophic	++	+

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Table 3

The identification of Chaperonin 10 by mass spectrometry and Western blotting techniques in malignant endometrial tissue homogeneates.

Case	Histopathologic Classification	MS results (relative intensity)	Western Blotting Results (relative intensity)
24	Em AdCa 1/3	++	++
25	Em AdCa 1/3	O	+
26	MMMT	++++	++++
27	Em AdCa	++	++
28	MMMT*	+	o
29	Em AdCa 2/3	+	+++
30	Em AdCa 2/3	+	++++
31	Ser AdCa	+	++++
32	Em AdCa 1/3	++++	++++
33	Em AdCa Grade n.k.	O	++++
34	Em AdCa Grade n.k.	+	+++
35	Em AdCa Grade n.k.	O	+
36	Em AdCa 1/3	+++++	++++
37	Em AdCa 1/3	++++	+++
38	Em AdCa 1/3	+++	+++
39	Muc AdCa 1/3	++	o
40	Em AdCa 1/3	+++	+++
41	Em AdCa 1/3	+++	++
42	Em AdCa – Ser AdCa	++++	++++
43	Em** AdCa 1/3	o	o
44	Em AdCa 2/3	++++	++++

*Mirror section showed minimal tumor.
**Mirror image showed necrotic tumor only.
Em AdCa = Endometrioid adenocarcinoma
MMMT = Malignant Mixed Mullerian Tumor
Muc AdCa = Mucinous adenocarcinoma
n.k. = not known
Ser AdCa = Serous adenocarcinoma

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Below full citations are set out for the references referred to in the specification.

Full Citations For References Referred To In The Specification

Example 1

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We Claim:

1. A method for detecting one or more endometrial markers or polynucleotides encoding the markers associated with an endometrial disease or an endometrium phase in a subject comprising:

   (a) obtaining a sample from a subject;

   (b) detecting in proteins extracted from the sample one or more endometrial markers or polynucleotides encoding the markers that are associated with the disease or phase; and

   (c) comparing the detected amount with an amount detected for a standard.

2. A method of detecting an endometrial disease in a subject, the method comprising comparing:

   (a) levels of one or more endometrial markers associated with endometrial disease that are extracted from a sample from the subject; and

   (b) normal levels of expression of the endometrial markers in a control sample, wherein a significant difference in levels of endometrial markers, relative to the corresponding normal levels, is indicative of endometrial disease.

3. A method as claimed in claim 1 or 2 comprising:

   (a) contacting a biological sample obtained from a subject with one or more binding agent that specifically binds to the endometrial markers or parts thereof; and

   (b) detecting in the sample amounts of endometrial markers that bind to the binding agents, relative to a predetermined standard or cut-off value, and therefrom determining the presence or absence of the endometrial disease in the subject.

4. A method as claimed in claim 3 wherein the binding agent is an antibody.

5. A method for screening a subject for endometrial cancer comprising (a) obtaining a biological sample from a subject; (b) detecting in proteins extracted from the sample the amount of one or more endometrial cancer markers; and (c) comparing the amount of endometrial cancer markers detected to a predetermined standard, where detection of a level of endometrial cancer markers different than

that of a standard is indicative of endometrial cancer.

6. A method as claimed in any preceding claim wherein the sample is obtained from tissues, extracts, cell cultures, cell lysates, lavage fluid, or physiological fluids.

7. A method as claimed in claim 6 wherein the sample is obtained from a tumor tissue.

8. A method as claimed in any preceding claim which further comprises detecting multiple cancer markers.

9. A method for determining the presence or absence of endometrial markers associated with an endometrial disease in a subject comprising detecting one or more polynucleotide encoding an endometrial marker in a sample from the subject and relating the detected amount to the presence of an endometrial disease.

10. A method as claimed in claim 9 wherein the polynucleotide detected is mRNA.

11. A method of claim 10 wherein the polynucleotide is detected by

(a) contacting the sample with oligonucleotides that hybridize to the polynucleotides; and

(b) detecting in the sample levels of nucleic acids that hybridize to the polynucleotides relative to a predetermined standard or cut-off value, and therefrom determining the presence or absence of an endometrial disease in the subject.

12. A method as claimed in claim 11 wherein the mRNA is detected using an amplification reaction.

13. A method as claimed in claim 12 wherein the amplification reaction is a polymerase chain reaction employing oligonucleotide primers that hybridize to the polynucleotides , or complements of such polynucleotides.

14. A method as claimed in claim 13 wherein the mRNA is detected using a hybridization technique employing oligonucleotide probes that hybridize to the polynucleotides or complements of such polynucleotides.

15. A method as claimed in claim 13 wherein the mRNA is detected by (a) isolating mRNA from the sample and combining the mRNA with reagents to convert it to cDNA; (b) treating the converted cDNA with amplification reaction reagents and primers that hybridize to the polynucleotides, to produce amplification products;

(d) analyzing the amplification products to detect an amount of mRNA encoding one or more endometrial cancer markers; and (e) comparing the amount of mRNA to an amount detected against a panel of expected values for normal and malignant tissue derived using similar primers.

16. A method for diagnosing and monitoring endometrial cancer in a subject comprising isolating nucleic acids in a sample from the subject; and detecting nucleic acids encoding endometrial cancer markers in the sample wherein the presence of higher or lower levels of nucleic acids encoding endometrial cancer markers in the sample compared to a standard or control is indicative of disease or prognosis.

17. A method for monitoring the progression of endometrial cancer in a subject, the method comprising: (a) detecting in a sample from the subject at a first time point, one or more endometrial cancer markers or polynucleotides encoding the markers; (b) repeating step (a) at a subsequent point in time; and (c) comparing levels detected in steps (a) and (b), and thereby monitoring the progression of endometrial cancer.

18. A method for determining in a subject whether endometrial cancer has metastasized or is likely to metastasize in the future, the method comprising comparing (a) levels of one or more endometrial cancer markers or polynucleotides encoding the markers, in a subject sample; and (b) normal levels or non-metastatic levels of the endometrial cancer markers or polynucleotides encoding the markers, in a control sample wherein a significant difference between the levels of expression in the subject sample and the normal levels or non-metastatic levels is an indication that the endometrial cancer has metastasized.

19. A method for assessing the aggressiveness or indolence of endometrial cancer comprising comparing: (a) levels of expression of one or endometrial cancer markers or polynucleotides encoding the markers, in a subject sample; and (b) normal levels of expression of the endometrial cancer markers or polynucleotides encoding the markers, in a control sample, wherein a significant difference between the levels in the subject sample and normal levels is an indication that the cancer is aggressive or indolent.

20. A diagnostic composition comprising an agent that binds to an endometrial cancer marker or hybridizes to a polynucleotide encoding such marker.

21. A method for assessing the potential efficacy of a test agent for inhibiting endometrial cancer in a subject, the method comprising comparing: (a) levels of one or more endometrial cancer markers, in a first sample obtained from a subject and exposed to the test agent, wherein the endometrial cancer markers, and (b) levels of the endometrial cancer markers in a second sample obtained from the subject, wherein the sample is not exposed to the test agent, wherein a significant difference in the levels of expression of the endometrial cancer markers in the first sample, relative to the second sample, is an indication that the test agent is potentially efficacious for inhibiting endometrial cancer in the subject.

22. A method of assessing the efficacy of a therapy for inhibiting endometrial cancer in a subject, the method comprising comparing: (a) levels of one or more endometrial cancer markers in a first sample obtained from the subject; and (b) levels of the endometrial cancer markers in a second sample obtained from the subject following therapy, wherein a significant difference in the levels of expression of the endometrial cancer markers in the second sample, relative to the first sample, is an indication that the therapy is efficacious for inhibiting endometrial cancer in the subject.

23. A method of selecting an agent for inhibiting endometrial cancer in a subject the method comprising (a) obtaining a sample comprising cancer cells from the subject; (b) separately exposing aliquots of the sample in the presence of a plurality of test agents; (c) comparing levels of one or more endometrial cancer markers in each of the aliquots; and (d) selecting one of the test agents which alters the levels of endometrial cancer markers in the aliquot containing that test agent, relative to other test agents.

24. A method of inhibiting endometrial cancer in a subject, the method comprising (a) obtaining a sample comprising cancer cells from the subject; (b) separately maintaining aliquots of the sample in the presence of a plurality of test agents; (c) comparing levels of one or more endometrial cancer markers in each of the aliquots; and (d) administering to the subject at least one of the test agents which alters the levels of endometrial cancer markers in the aliquot containing that test

agent, relative to other test agents.

25. A method of assessing the endometrial cancer cell carcinogenic potential of a test compound, the method comprising: (a) maintaining separate aliquots of endometrial cancer cells in the presence and absence of the test compound; and (b) comparing expression of one or more endometrial cancer markers, in each of the aliquots, and wherein a significant difference in levels of endometrial cancer markers in the aliquot maintained in the presence of the test compound, relative to the aliquot maintained in the absence of the test compound, is an indication that the test compound possesses endometrial cancer cell carcinogenic potential.

26. An *in vivo* method for imaging an endometrial disease comprising:

(a) injecting a subject with one or more agent that binds to an endometrial marker, the agent carrying a label for imaging the endometrial marker;

(b) allowing the agent to incubate *in vivo* and bind to an endometrial marker; and

(c) detecting the presence of the label localized to the endometrial cancer.

27. A method as claimed in claim 26 wherein the agent is an antibody that specifically reacts with an endometrial marker.

28. A method of any preceding claim wherein the endometrial markers are one or more of the proteins listed in Table 1.

29. A method of determining uterine endometrial receptivity by first obtaining a serum, uterine fluid or endometrial biopsy sample from a subject and detecting the presence of an endometrial marker associated with a certain endometrium phase, wherein the presence or absence of an endometrial marker as compared to controls indicates uterine receptivity.

30. A method of claim 29 wherein the endometrium phase is the secretory or proliferative phase.

31. A method of monitoring the effects of ovarian hyperstimulation and/or ovulation induction protocols on uterine receptivity which comprises: (a) obtaining a serum, uterine or fluid or endometrial biopsy sample from a subject receiving the treatments; and (b) detecting the presence of an endometrial marker present in the endometrium at the time of fertilization, early embryogenesis, and implantation; wherein presence or absence of an endometrial marker indicates receptivity.

32. A method of determining a probability of successful implantation with an ovarian stimulation *in vitro* fertilization and embryo transfer procedure, comprising:

(a) determining a level of an endometrial marker in a sample obtained from a subject who has undergone an ovarian stimulation *in vitro* fertilization and embryo transfer procedure; and

(b) determining a probability of successful implantation based on the subject's determined endometrial marker level;

wherein a significantly different endometrial marker level relative to a standard level is associated with a decreased or increased probability of successful implantation.

33. A method of any of claim 29 to 32 wherein the endometrial marker is glutamate receptor subunit zeta 1, a tryptic fragment thereof, and/or macrophage migration inhibitory factor.

34. A method of contraception by interrupting the cyclic presence of an endometrial marker, in particular glutamate receptor subunit zeta 1, a tryptic fragment thereof, and/or macrophage migration inhibitory factor.

35. A kit for carrying out a method as claimed in any preceding claim.

36. A kit for determining the presence of an endometrial disease in a subject, comprising a known amount of one or more binding agent that specifically binds to an endometrial marker wherein the binding agent comprises a detectable substance, or it binds directly or indirectly to a detectable substance.

37. A kit for determining the presence of endometrial disease in a subject, comprising a known amount of an oligonucleotide that hybridizes to a polynucleotide encoding an endometrial marker wherein the oligonucleotide is directly or indirectly labeled with a detectable substance.

ABSTRACT OF THE DISCLOSURE

Methods for detecting endometrial diseases or an endometrium phase in a subject are described comprising measuring endometrial markers or polynucleotides encoding the markers in a sample from the subject. The invention also provides localization or imaging methods for endometrial diseases, and kits for carrying out the methods of the invention. The invention also contemplates therapeutic applications for endometrial diseases employing endometrial markers, polynucleotides encoding the markers, and/or binding agents for the markers.

Sequence Listing

SEQ ID NO. 1

Chaperonin 10

Q04984

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61 qpvsvkvgdk vllpeyggtk vvlddkdyfl frdgdilgky vd
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SEQ ID NO. 2

NM_002157 and HSU07550

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 61 gaaagtttct tccactcttt gaccgagtat tggttgaaag gagtgctgct gaaactgtaa
121 ccaaaggagg cattatgctt ccagaaaaat ctcaaggaaa agtattgcaa gcaacagtag
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421 tgtaaataat ttccatattt ctcttttata ataaactaat gataactaat gacatccagt
481 gtctccaaaa ttgtttcctt gtactgatat aaacacttcc aaataaaaat atgtaaat
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SEQ ID NO. 3

Calgranulin A

P05109

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61 ntdgavnfqe flilvikmgv aahkkshees hke
```

SEQ ID NO. 4

NM_002964 and BT00378

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 61 gaccgagctg gagaaagcct tgaactctat catcgacgtc taccacaagt actccctgat
121 aaaggggaat ttccatgccg tctacaggga tgacctgaag aaattgctag agaccgagtg
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241 tgatggtgca gttaacttcc aggagttcct cattctggtg ataaagatgg gcgtggcagc
301 ccacaaaaaa agccatgaag aaagccacaa agagtagctg agttactggg cccagaggct
361 gggcccctgg acatgtacct gcagaataat aaagtcatca atacctcaaa aaaaaaaaaa
421 aaaaaaaa
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SEQ ID NO. 5

Calgranulin B/MRP-14

P06702

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SEQ ID NO. 6

X06233

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gccacggcca cagtcatggt ggccacggcc acagccaccc at

SEQ ID NO. 7

Polymeric-immunoglobulin receptor (precursor)

P01833 or Q81ZY7

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121 fdvslevsqg pgllndtkvy tvdlgrtvti ncpfktenaq krkslykqig lypvlvidss
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SEQ ID NO. 8

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4141 tgctctccag aaagctaaaa tttaatttct tttttcctct gagttctgta cttcaaccag
4201 cctacaagct ggcacttgct aacaaatcag aaatatgaca attaatgatt aaagactgtg
4261 attgcc
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SEQ ID NO. 9

Phosphatidylethanolamine binding protein (PEBP)

P30086

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181 yeqlsgk
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SEQ ID NO. 10

BC031102

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 241 cagcatttcg tgggatggtc ttgattcagg gaagctctac accttggtcc tgacagaccc
 301 ggatgctccc agcaggaagg atcccaaata cagagaatgg catcatttcc tggtggtcaa
 361 catgaagggc aatgacatca gcagtggcac agtcctctcc gattatgtgg gctcggggcc
 421 tcccaagggc acaggcctcc accgctatgt ctggctggtt tacgagcagg acaggccgct
 481 aaagtgtgac gagcccatcc tcagcaaccg atctggagac caccgtggca aattcaaggt
 541 ggcgtccttc cgtaaaaagt atgagctcag ggccccggtg gctggcacgt gttaccaggc
 601 cgagtgggat gactatgtgc ccaaactgta cgagcagctg tctgggaagt agggggttag
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1321 tttttaaatt gatcgttctt catgggggta agaaaagctg gtctggagtt gctgaatgtt
1381 gcattaattg tgctgtttgc ttgtagttga ataaaaatag aaacctgaat gaagaaaaaa
1441 aaaaaaaaaa
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SEQ ID NO. 11

Acidic leucine-rich nuclear phosphoprotein 32 family member A

P39687

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  1 memgrrihle lrnrtpsdvk elvldnsrsn egklegltde feeleflsti nvgltsianl
 61 pklnklkkle lsdnrvsggl evlaekcpnl thlnlsgnki kdlstieplk klenlksldl
121 fncevtnlnd yrenvfkllp qltyldgydr ddkeapdsda egyvegldde eededeeeyd
181 edaqvvedee dedeeeegee edvsgeeeed eegyndgevd deedeeelge eergqkrkre
241 pedegeddd
```

SEQ ID NO. 12

X75090

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  1 gctggttgag ccttcaaagt cctaaaacgc gcggccgtgg gttcggggtt tattgattga
 61 attccgccgg cgcgggagcc tctgcagaga gagagcgcga gagatggaga tgggcagacg
121 gattcattta gagctgcgga acaggacgcc ctctgatgtg aaagaacttg tcctggacaa
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  181 cagtcggtcg aatgaaggca aactcgaagg cctcacagat gaatttgaag aactggaatt
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  301 taagaagctt gaactaagcg ataacagagt ctcagggggc ctggaagtat tggcagaaaa
  361 gtgtccgaac ctcacgcatc taaatttaag tggcaacaaa attaaagacc tcagcacaat
  421 agagccactg aaaaagttag aaaacctcaa gagcttagac cttttcaatt gcgaggtaac
  481 caacctgaac gactaccgag aaaatgtgtt caagctcctc ccgcaactca catatctcga
  541 cggctatgac cgggacgaca aggaggcccc tgactcggat gctgagggct acgtggaggg
  601 cctggatgat gaggaggagg atgaggatga ggaggagtat gatgaagatg ctcaggtagt
  661 ggaagacgag gaggacgagg atgaggagga ggaaggtgaa gaggaggacg tgagtggaga
  721 ggaggaggag gatgaagaag gttataacga tggagaggta gatgacgagg aagatgaaga
  781 agagcttggt gaagaagaaa ggggtcagaa gcgaaaacga gaacctgaag atgagggaga
  841 agatgatgac taagtggaat aacctatttt gaaaaattcc tattgtgatt tgactgtttt
  901 tacccatatc ccctct
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SEQ ID NO. 13

Heat shock 70kDa protein

P17066

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    1 mqaprelavg idlgttyscv gvfqqgrvei landqgnrtt psyvaftdte rlvgdaaksq
   61 aalnphntvf dakrligrkf adttvqsdmk hwpfrvvseg gkpkvrvcyr gedktfypee
  121 issmvlskmk etaeaylgqp vkhavitvpa yfndsqrqat kdagaiagln vlriinepta
  181 aaiaygldrr gagernvlif dlgggtfdvs vlsidagvfe vkatagdthl ggedfdnrlv
  241 nhfmeefrrk hgkdlsgnkr alrrlrtace rakrtlssst qatleidslf egvdfytsit
  301 rarfeelcsd lfrstlepve kalrdakldk aqihdvvlvg gstripkvqk llqdffngke
  361 lnksinpdea vaygaavqaa vlmgdkcekv qdlllldvap lslgletagg vmttliqrna
  421 tiptkqtqtf ttysdnqpgv fiqvyegera mtkdnnllgr felsgippap rgvpqievtf
  481 didangilsv tatdrstgka nkititndkg rlskeeverm vheaeqykae deaqrdrvaa
  541 knsleahvfh vkgslqeesl rdkipeedrr kmqdkcrevl awlehnqlae keeyehqkre
  601 leqicrpifs rlyggpgvpg gsscgtqarq gdpstgpiie evd
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SEQ ID NO. 14

NM_002155

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  121 acgggagctc gcggtgggca tcgacctggg caccacctac tcgtgcgtgg gcgtgtttca
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  241 ggccttcacc gacaccgagc ggctggtcgg ggacgcggcc aagagccagg cggccctgaa
  301 cccccacaac accgtgttcg atgccaagcg gctgatcggg cgcaagttcg cggacaccac
  361 ggtgcagtcg gacatgaagc actggccctt ccgggtggtg agcgagggcg gcaagcccaa
  421 ggtgcgcgta tgctaccgcg gggaggacaa gacgttctac cccgaggaga tctcgtccat
  481 ggtgctgagc aagatgaagg agacggccga ggcgtacctg ggccagcccg tgaagcacgc
  541 agtgatcacc gtgcccgcct atttcaatga ctcgcagcgc caggccacca aggacgcggg
  601 ggccatcgcg gggctcaacg tgttgcggat catcaatgag cccacggcag ctgccatcgc
  661 ctatgggctg gaccggcggg gcgcgggaga gcgcaacgtg ctcatttttg acctgggtgg
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  781 tgctggagat acccacctgg gaggagagga cttcgacaac cggctcgtga accacttcat
  841 ggaagaattc cggcggaagc atgggaagga cctgagcggg aacaagcgtg ccctgcgcag
  901 gctgcgcaca gcctgtgagc gcgccaagcg caccctgtcc tccagcaccc aggccaccct
  961 ggagatagac tccctgttcg agggcgtgga cttctacacg tccatcactc gtgcccgctt
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 1141 catccccaag gtgcagaagt tgctgcagga cttcttcaac ggcaaggagc tgaacaagag
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1381 caagcagacc cagactttca ccacctactc ggacaaccag cctggggtct tcatccaggt
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1621 catcaccaat gacaagggcc ggctgagcaa ggaggaggtg gagaggatgg ttcatgaagc
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1981 tggcactcaa gcccgccagg gggaccccag caccggcccc atcattgagg aggttgattg
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2101 tgtcttctat gatcctgccc ttcagagatg aactttccct ccaaagctag aactttcttc
2161 ccaggataac tgaagtcttt tgacttttg gggggagggc ggttcatcct cttctgcttc
2221 aaataaaaag tcattaattt aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaa
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SEQ ID NO. 15

P14174

macrophage migration inhibitory factor

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 1 mpmfivntnv prasvpdgfl seltqqlaqa tgkppqyiav hvvpdqlmaf ggssepcalc
61 slhsigkigg aqnrsyskll cgllaerlri spdrvyinyy dmnaanvgwn nstfa
```

SEQ ID NO. 16

NM_002415

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  1 accacagtgg tgtccgagaa gtcaggcacg tagctcagcg gcggccgcgg cgcgtgcgtc
 61 tgtgcctctg cgcgggtctc ctggtccttc tgccatcatg ccgatgttca tcgtaaacac
121 caacgtgccc cgcgcctccg tgccggacgg gttcctctcc gagctcaccc agcagctggc
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241 ggccttcggc ggctccagcg agccgtgcgc gctctgcagc ctgcacagca tcggcaagat
301 cggcggcgcg cagaaccgct cctacagcaa gctgctgtgc ggcctgctgg ccgagcgcct
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421 ctggaacaac tccaccttcg cctaagagcc gcagggaccc acgctgtctg cgctggctcc
481 acccgggaac ccgccgcacg ctgtgttcta ggcccgccca ccccaacctt ctggtgggga
541 gaaataaacg gtttagagac t
```

SEQ ID NO. 17

Calgizzarin

P31949

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 1 makissptet erciesliav fqkyagkdgy nytlsktefl sfmntelaaf tknqkdpgvl
61 drmmkkldtn sdgqldfsef lnligglama chdsflkavp sqkrt
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SEQ ID NO. 18

NM_005620

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   121 atggcaaaaa tctccagccc tacagagact gagcggtgca tcgagtccct gattgctgtc
   181 ttccagaagt atgctggaaa ggatggttat aactacactc tctccaagac agagttccta
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   421 tcccagaagc ggacctgagg acccccttggc cctggccttc aaacccaccc cctttccttc
   481 cagcctttct gtcatcatct ccacagccca cccatcccct gagcacacta accacctcat
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SEQ ID NO. 19

Triosephosphate isomerase

P00938

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     1 mapsrkffvg gnwkmngrkq slgeligtln aakvpadtev vcapptayid farqkldpki
    61 avaaqncykv tngaftgeis pgmikdcgat wvvlghserr hvfgesdeli gqkvahalae
   121 glgviacige kldereagit ekvvfeqtkv iadnvkdwsk vvlayepvwa igtgktatpq
   181 qaqevheklr gwlksnvsda vaqstriiyg gsvtgatcke lasqpdvdgf lvggaslkpe
   241 fvdiinakq
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SEQ ID NO. 20

NM_000365

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   121 gaacgcggcc aaggtgccgg ccgacaccga ggtggtttgt gctcccccta ctgcctatat
   181 cgacttcgcc cggcagaagc tagatcccaa gattgctgtg gctgcgcaga actgctacaa
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   661 tggaggctct gtgactgggg caacctgcaa ggagctggcc agccagcctg atgtggatgg
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   781 atgagcccca tccatcttcc ctacccttcc tgccaagcca gggactaagc agcccagaag
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  1141 aggcaggagt gctgccctct cccatggtgc ccgtgcctct gtgctgtgta tgtgaaccac
  1201 ccatgtgagg gaataaacct ggcactagga aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
  1261 aaa
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SEQ ID NO. 21

Q05586

Glutamate [NMDA] receptor subunit zeta 1 precursor

MSTMRLLTLA LLFSCSVARA ACDPKIVNIG AVLSTRKHEQ MFREAVNQAN KRHGSWKIQL
NATSVTHKPN AIQMALSVCE DLISSQVYAI LVSHPPTPND HFTPTPVSYT AGFYRIPVLG
LTTRMSIYSD KSIHLSFLRT VPPYSHQSSV WFEMMRVYSW NHIILLVSDD HEGRAAQKRL
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TGSGYVWLVG EREISGNALR YAPDGILGLQ LINGKNESAH ISDAVGVVAQ AVHELLEKEN
ITDPPRGCVG NTNIWKTGPL FKRVLMSSKY ADGVTGRVEF NEDGDRKFAN YSIMNLQNRK
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KEEFTVNGDP VKKVICTGPN DTSPGSPRHT VPQCCYGFCI DLLIKLARTM NFTYEVHLVA
DGKFGTQERV NNSNKKEWNG MMGELLSGQA DMIVAPLTIN NERAQYIEFS KPFKYQGLTI
LVKKEIPRST LDSFMQPFQS TLWLLVGLSV HVVAVMLYLL DRFSPFGRFK VNSEEEEEDA
LTLSSAMWFS WGVLLNSGIG EGAPRSFSAR ILGMVWAGFA MIIVASYTAN LAAFLVLDRP
EERITGINDP RLRNPSDKFI YATVKQSSVD IYFRRQVELS TMYRHMEKHN YESAAEAIQA
VRDNKLHAFI WDSAVLEFEA SQKCDLVTTG ELFFRSGFGI GMRKDSPWKQ NVSLSILKSH
ENGFMEDLDK TWVRYQECDS RSNAPATLTF ENMAGVFMLV AGGIVAGIFL IFIEIAYKRH
KDARRKQMQL AFAAVNVWRK NLQDRKSGRA EPDPKKKATF RAITSTLASS FKRRRSSKDT
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SEQ ID NO. 22

D13515

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SEQ ID NO. 23

LLTLLALLFSCSVAR

Figure 1

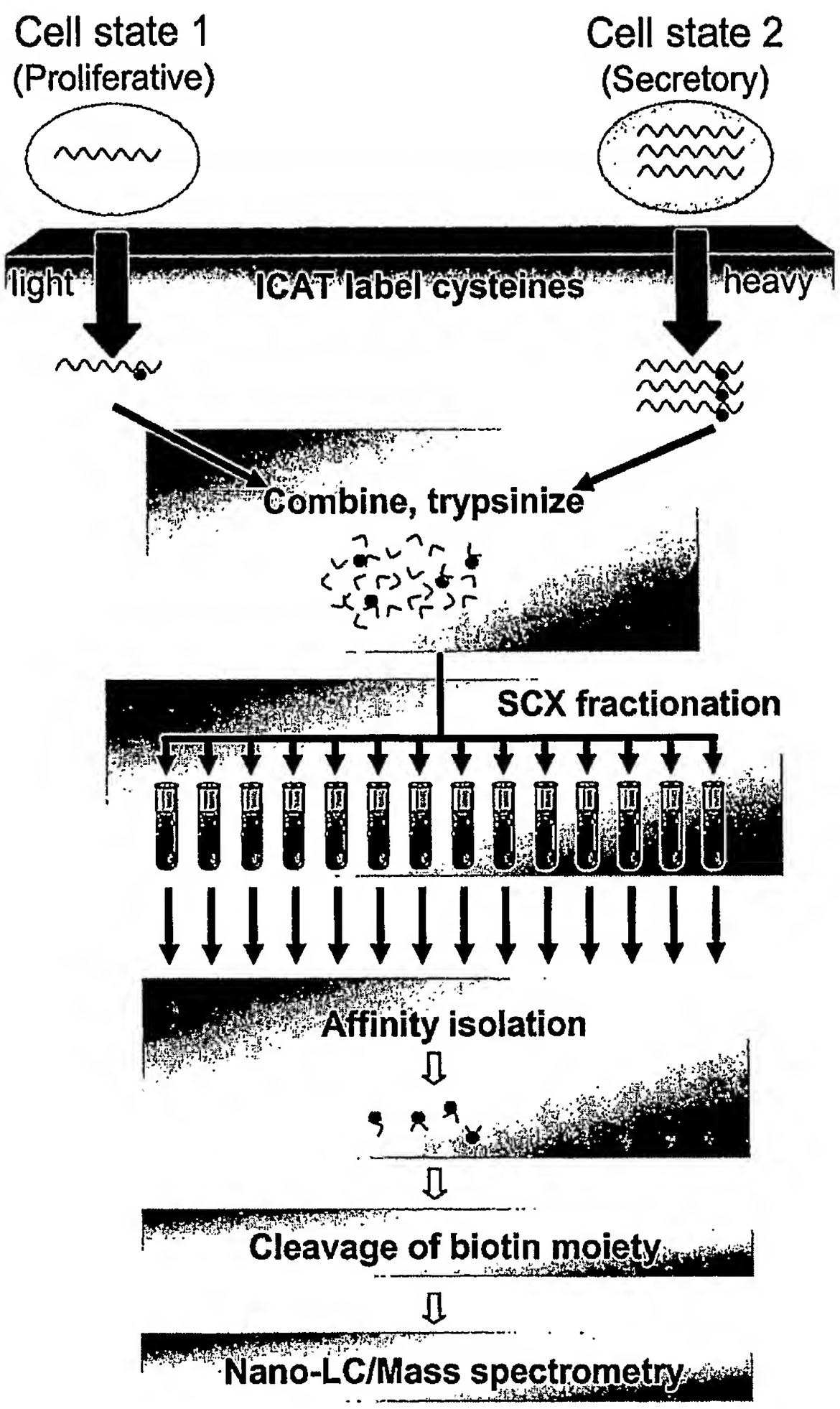
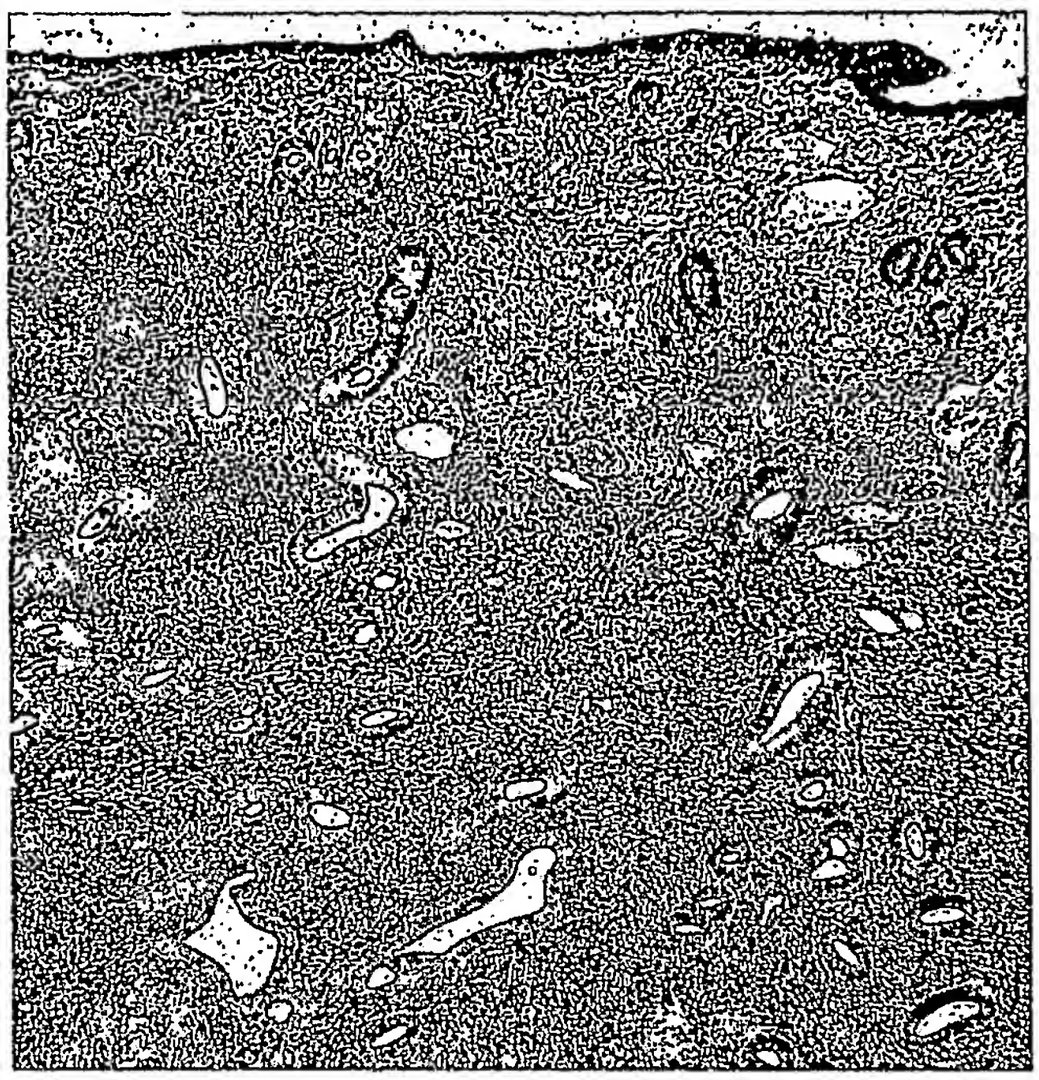


Figure 2

A



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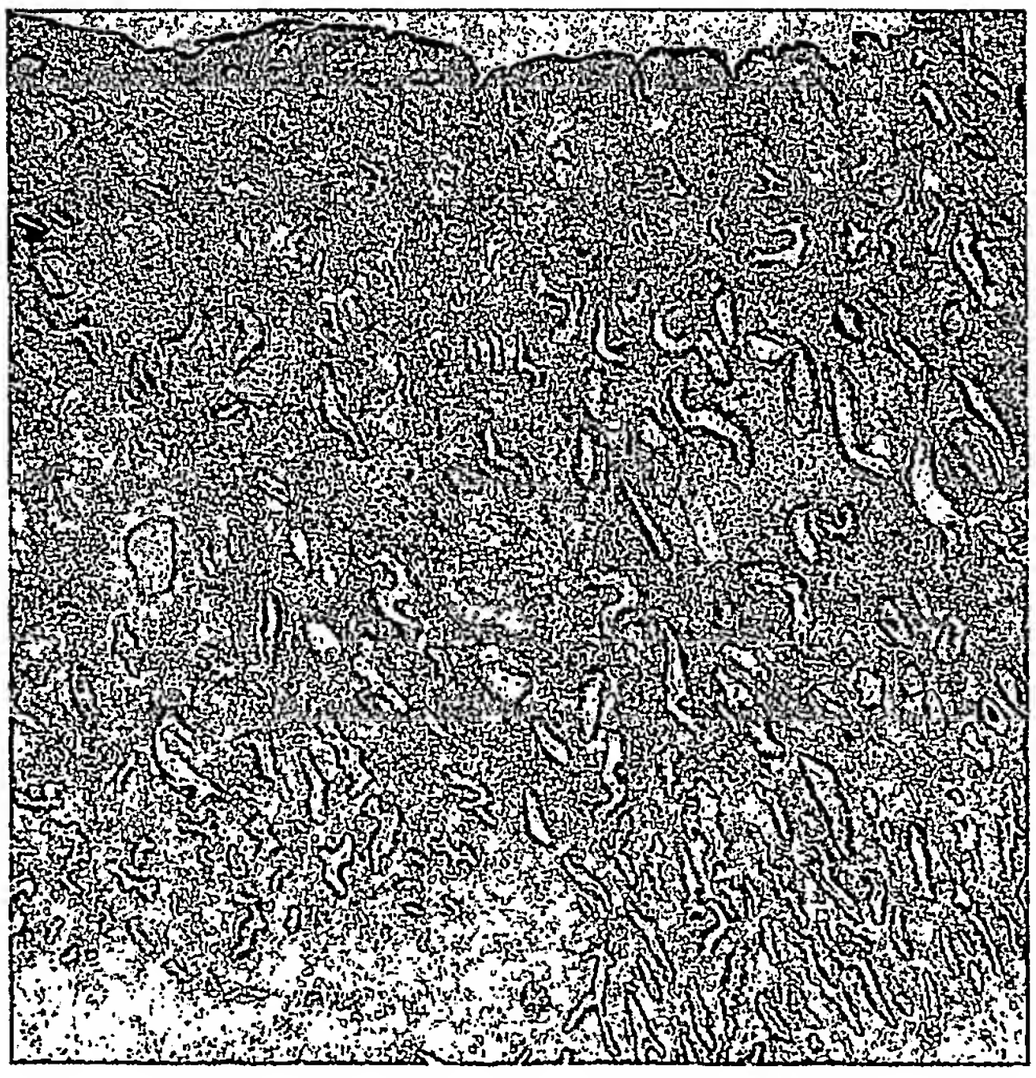

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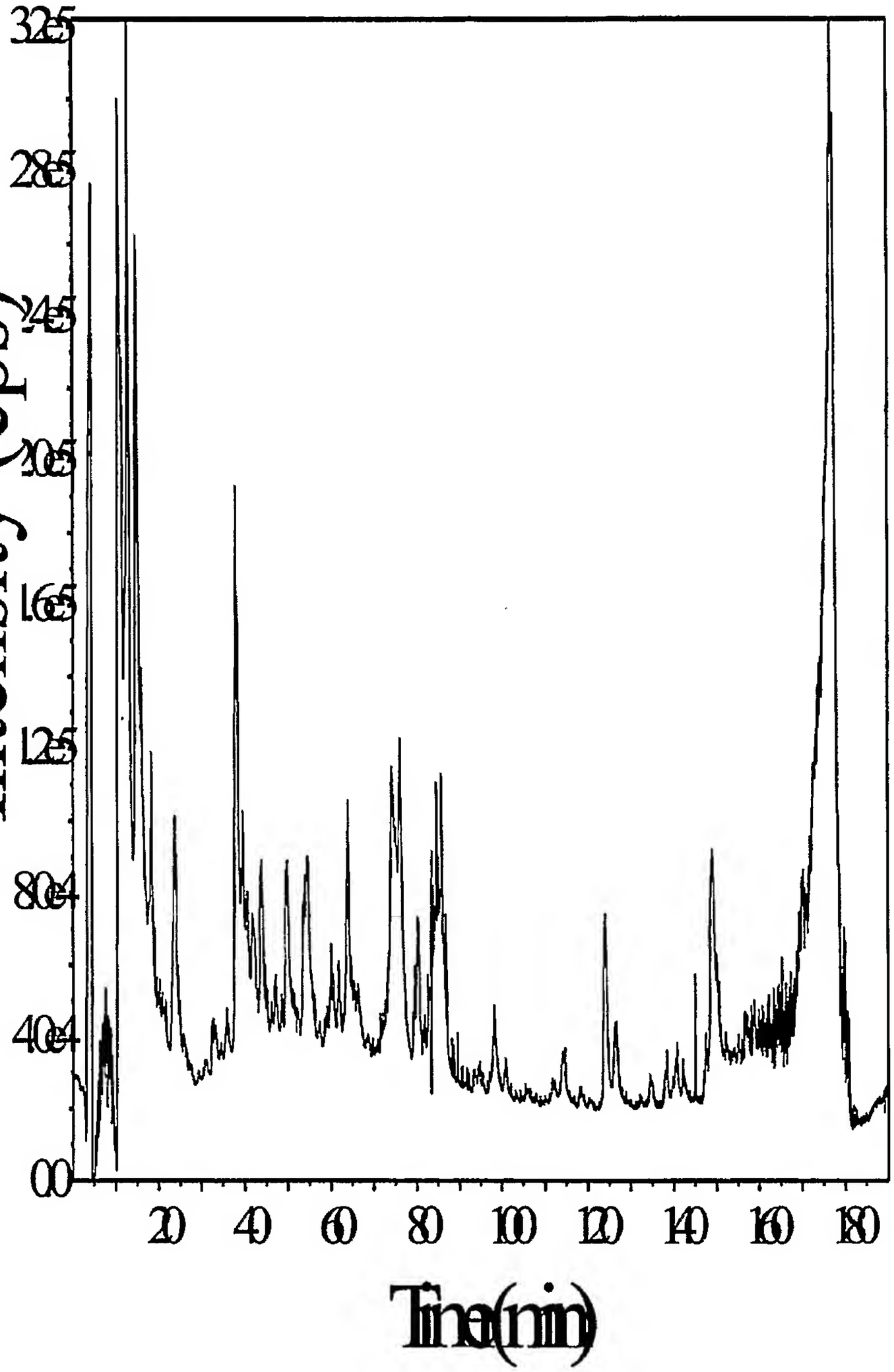

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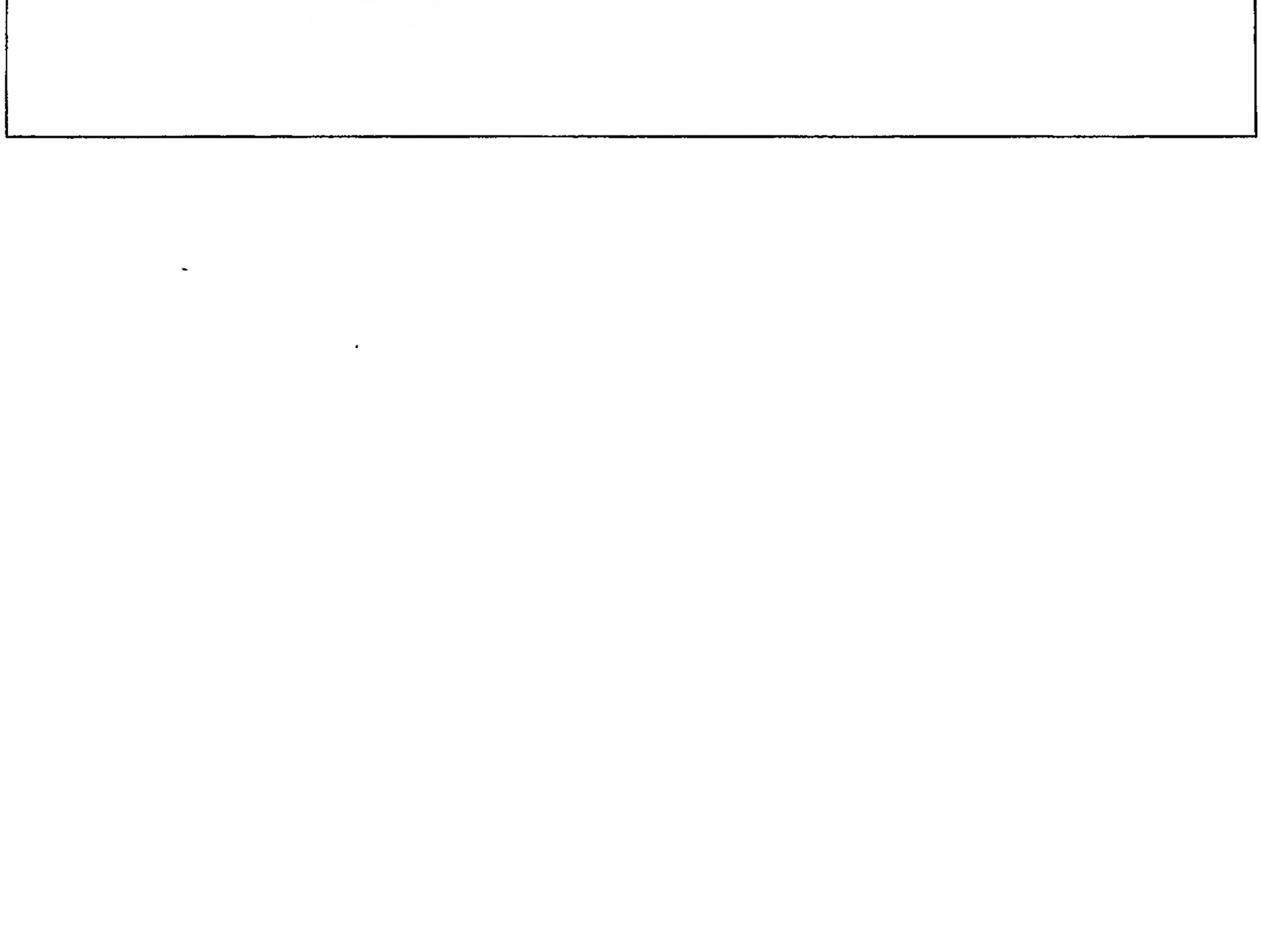

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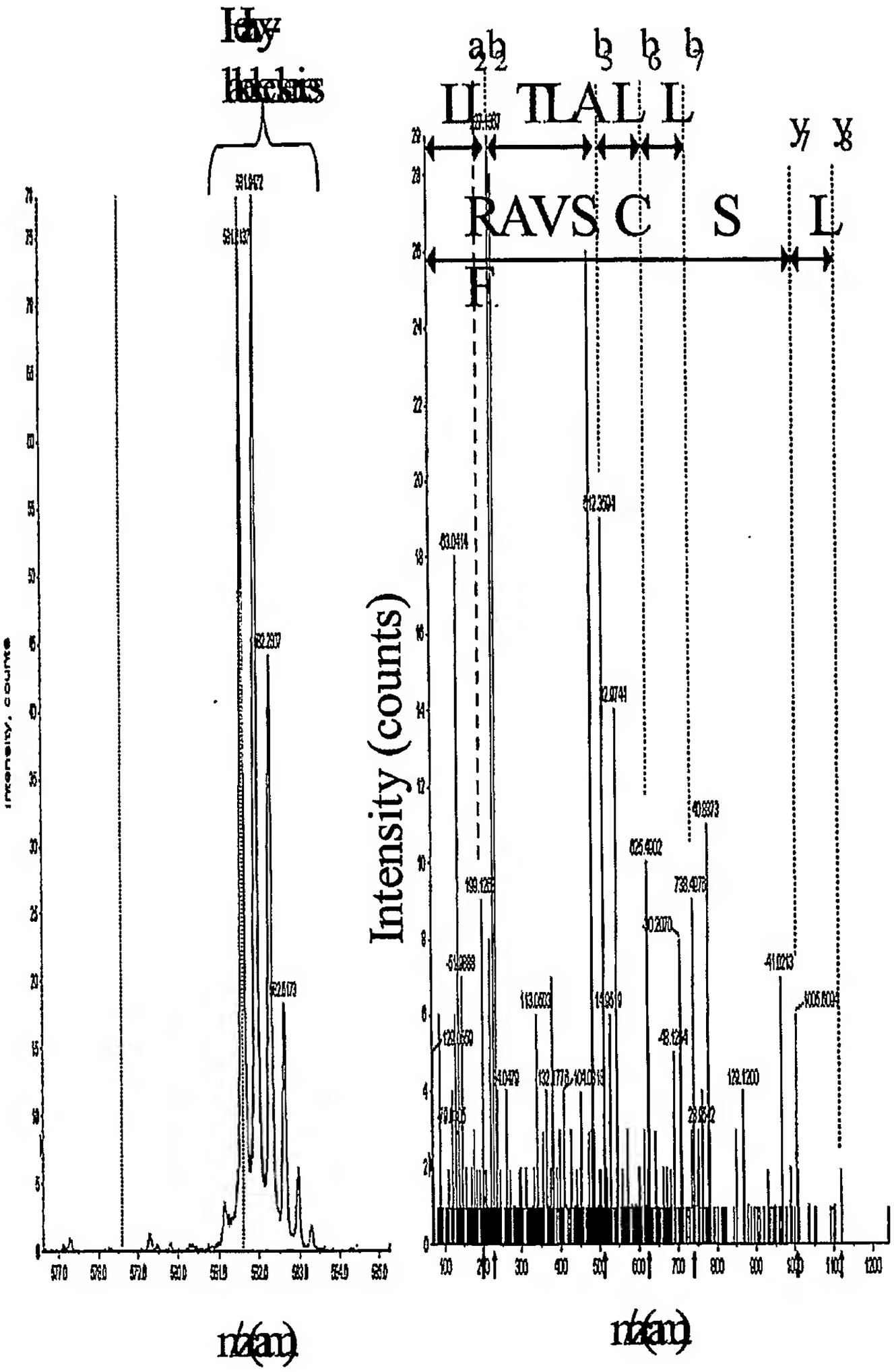

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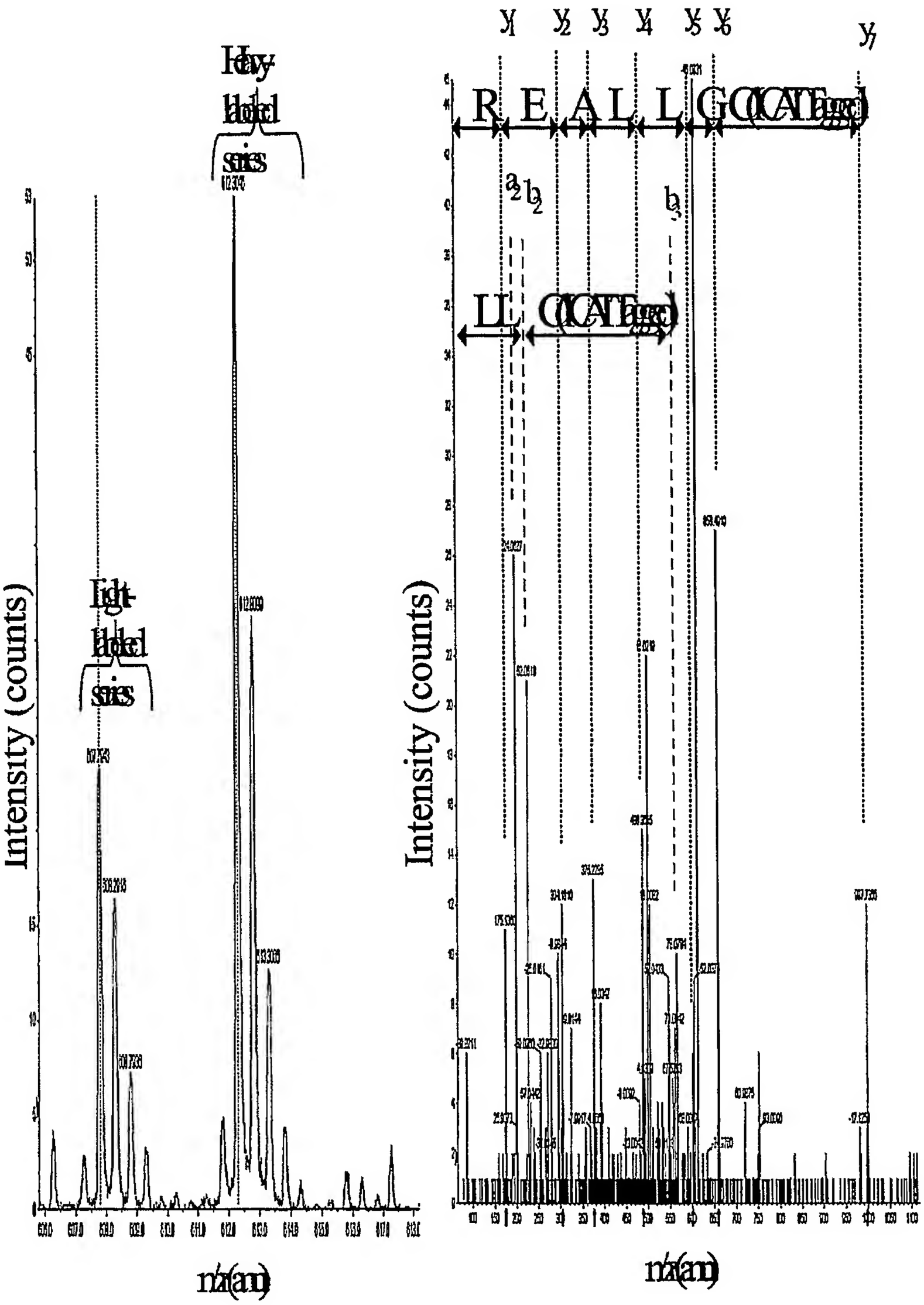

A **B**

Inventor: COLGAN ET AL.
Docket No.: 14096.36USP1
Title: ENDOMETRIAL MARKERS
Attorney Name: DOUGLAS P. MUELLER
Phone No.: 612.371.5237
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Figure 7

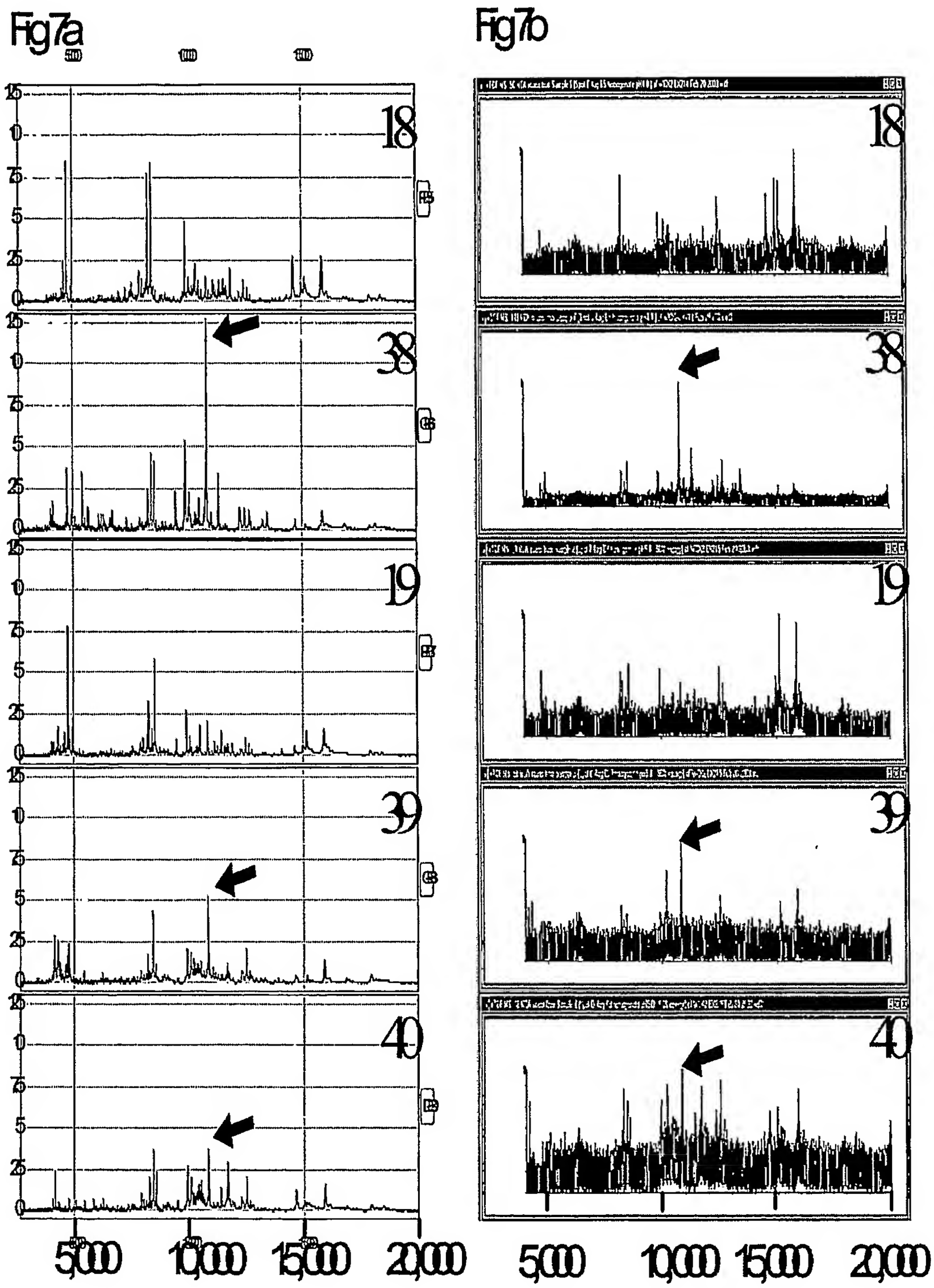
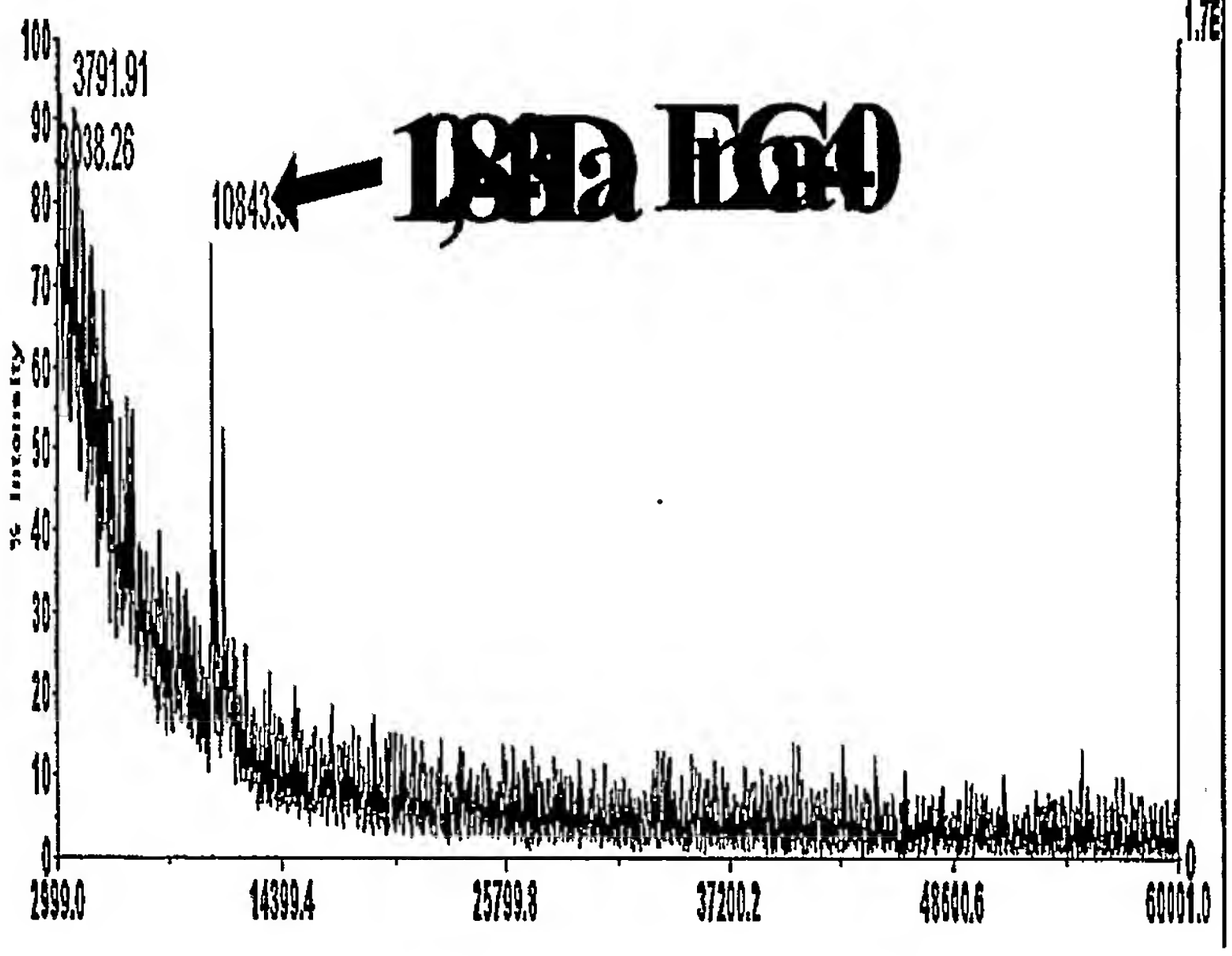


Figure 8A



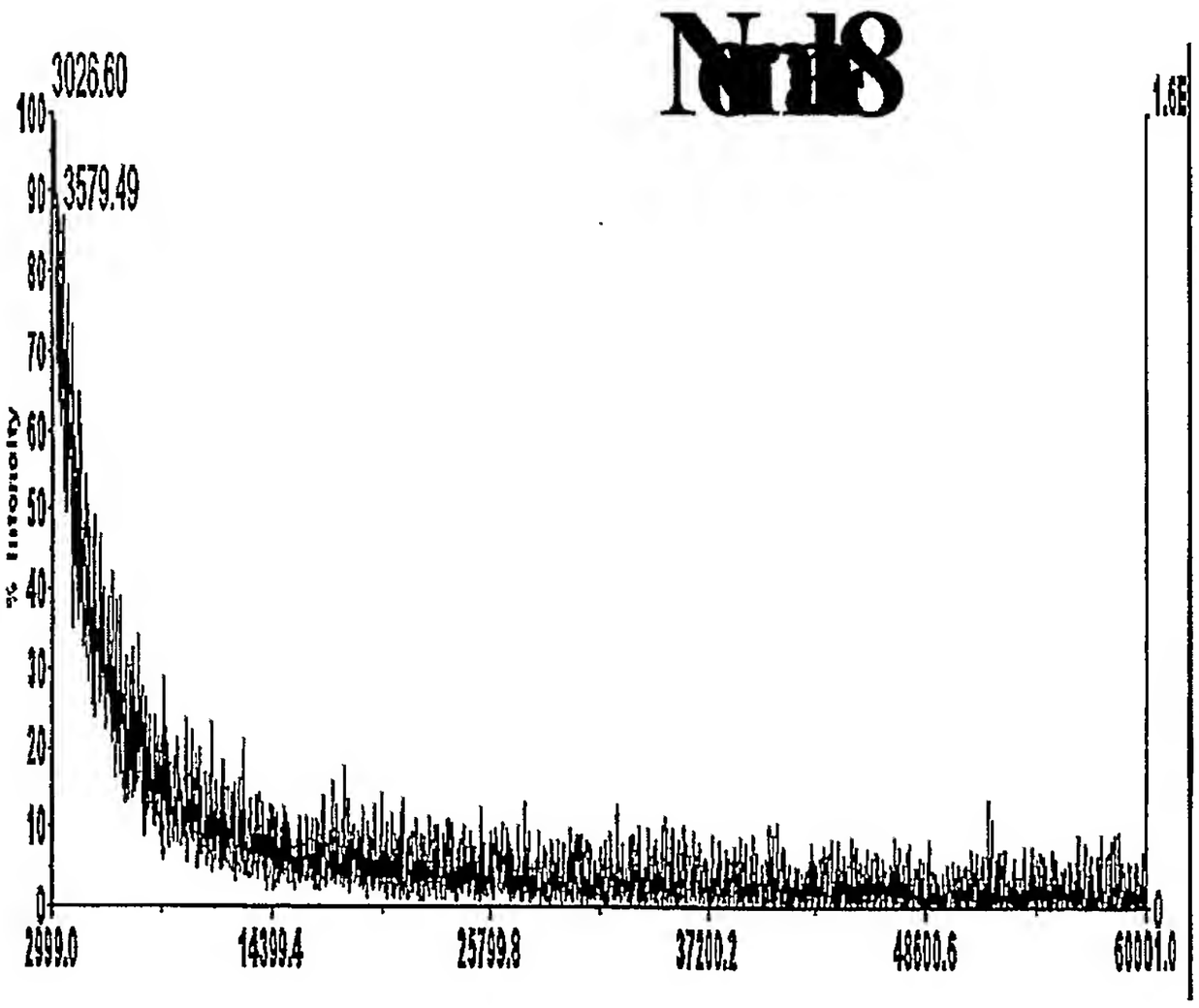
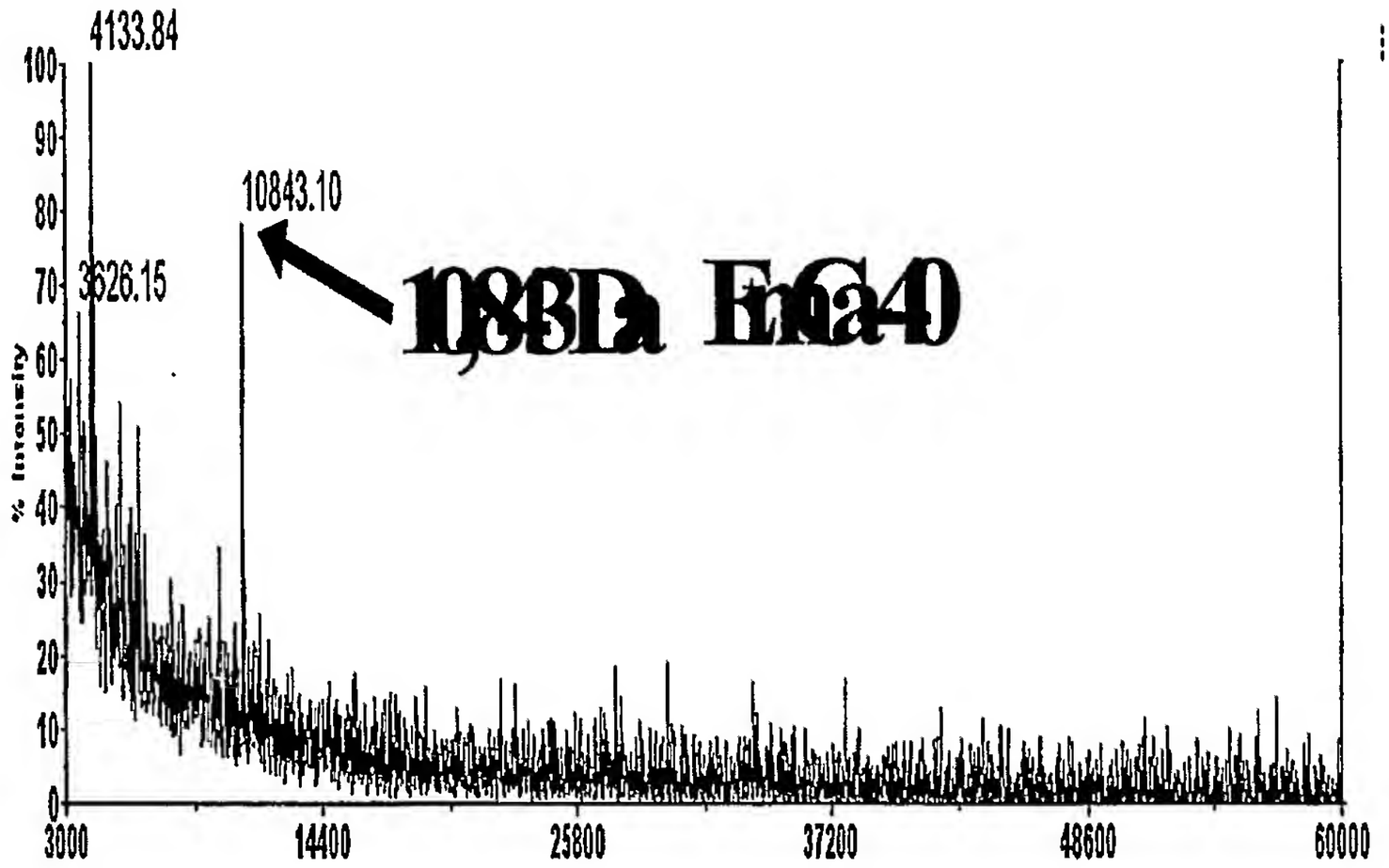


Figure 8B



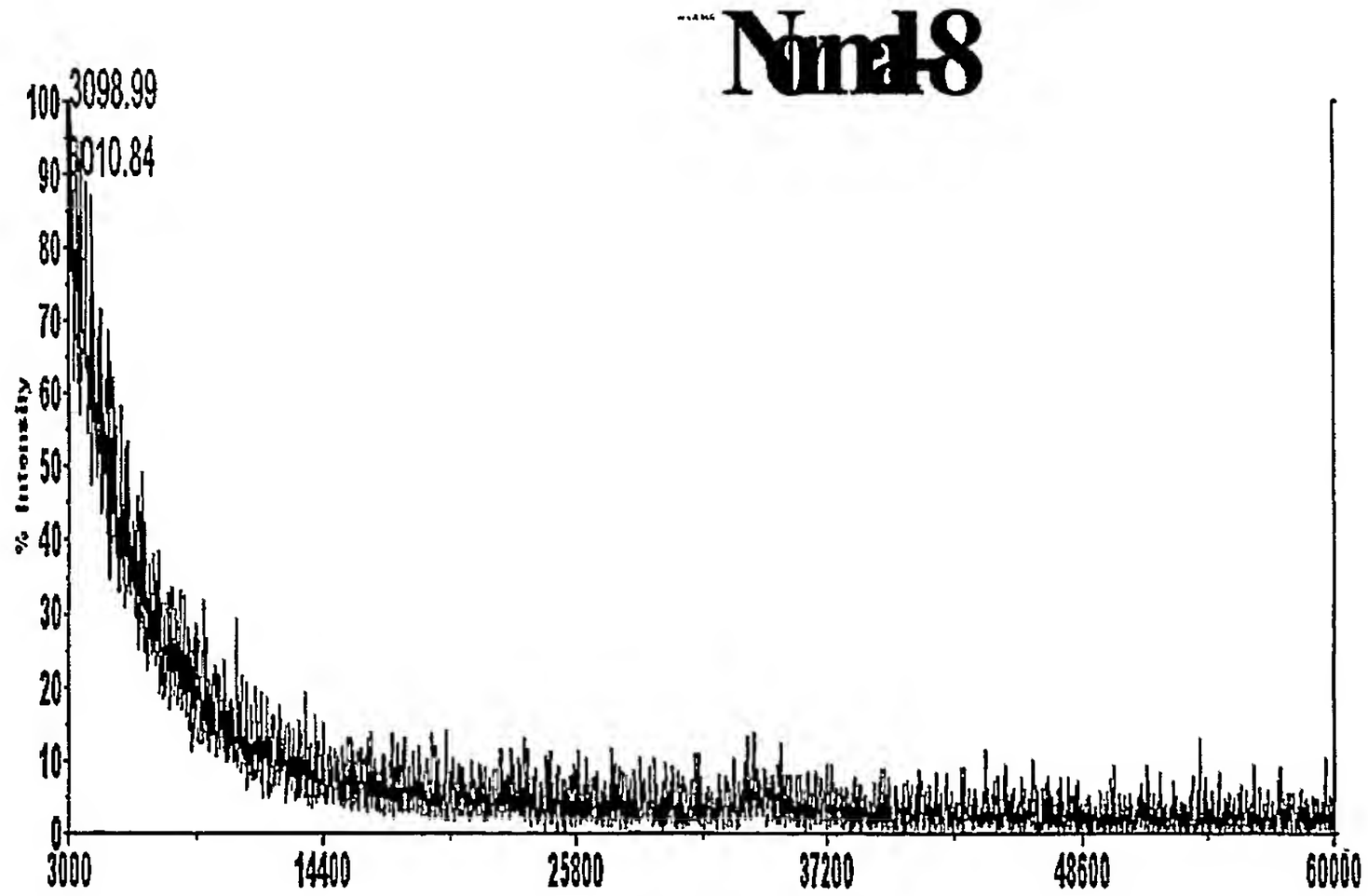


Figure 8C

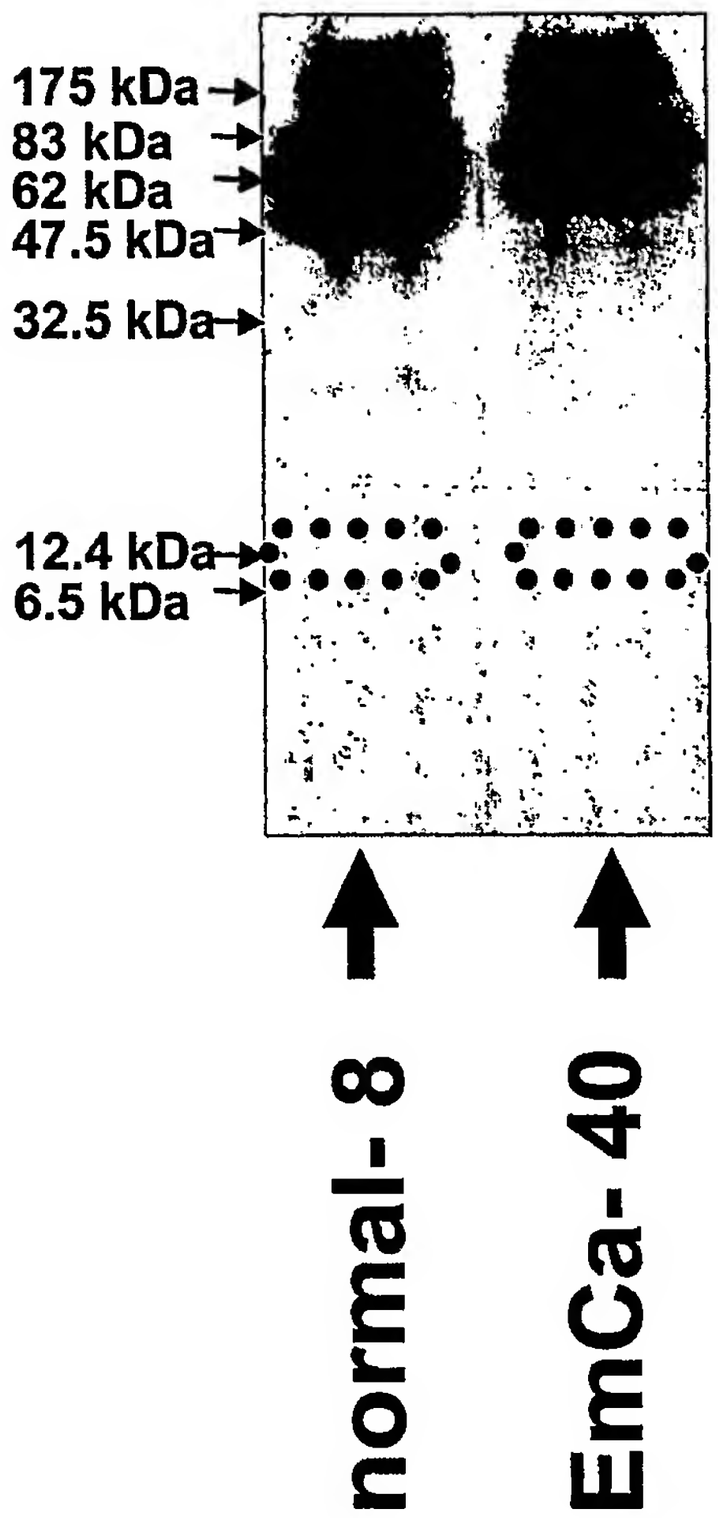


Figure 8D

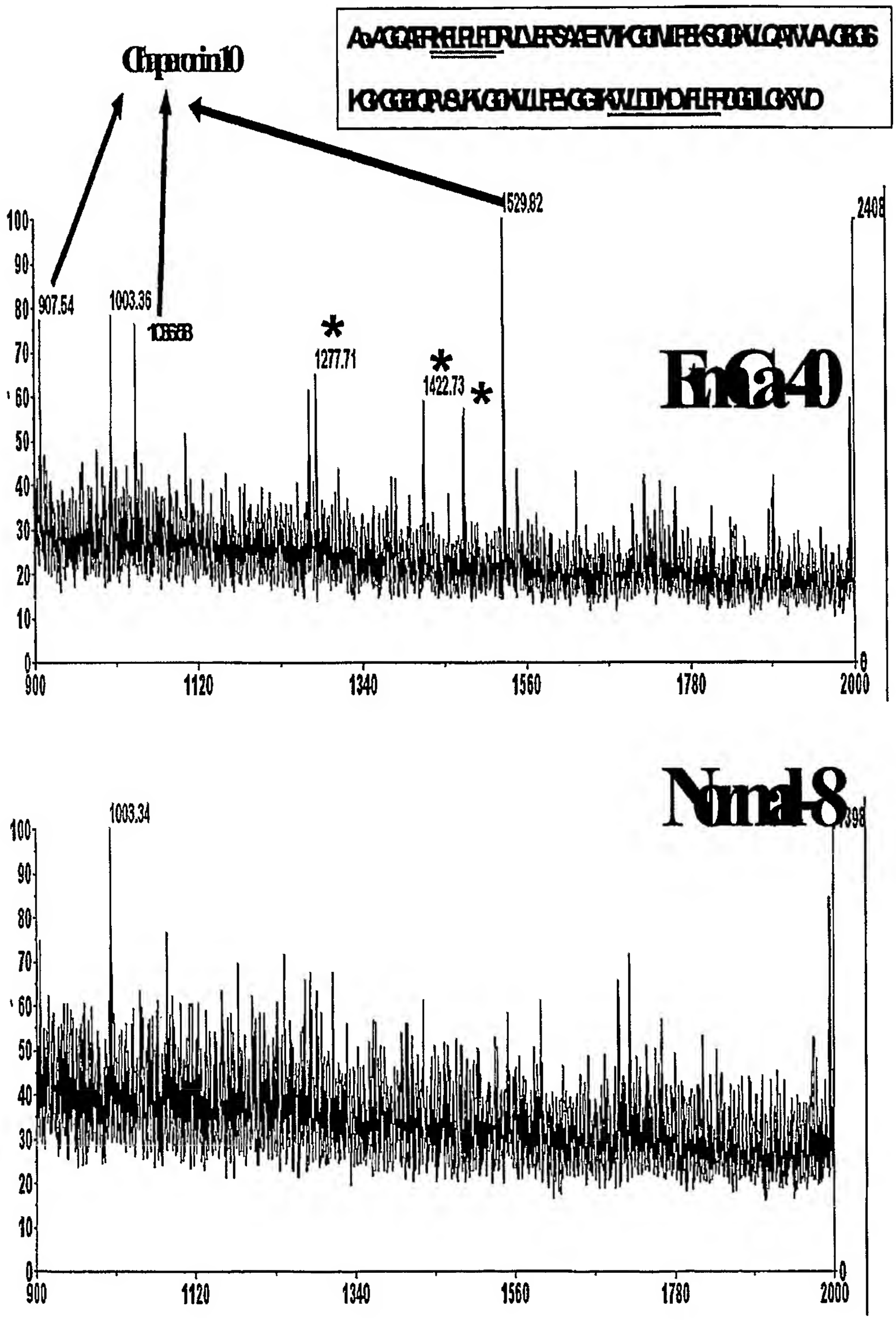
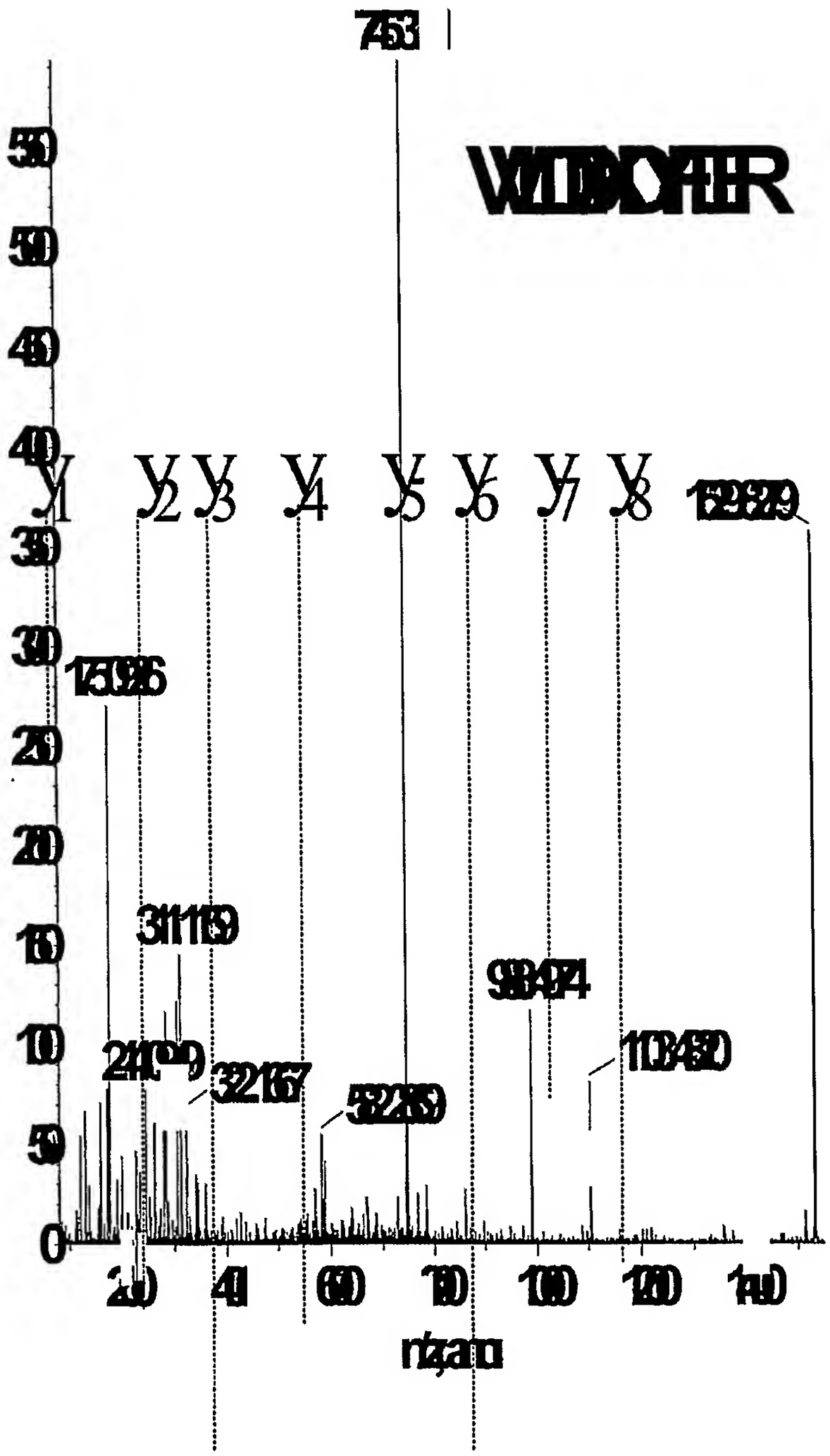


Figure 8E



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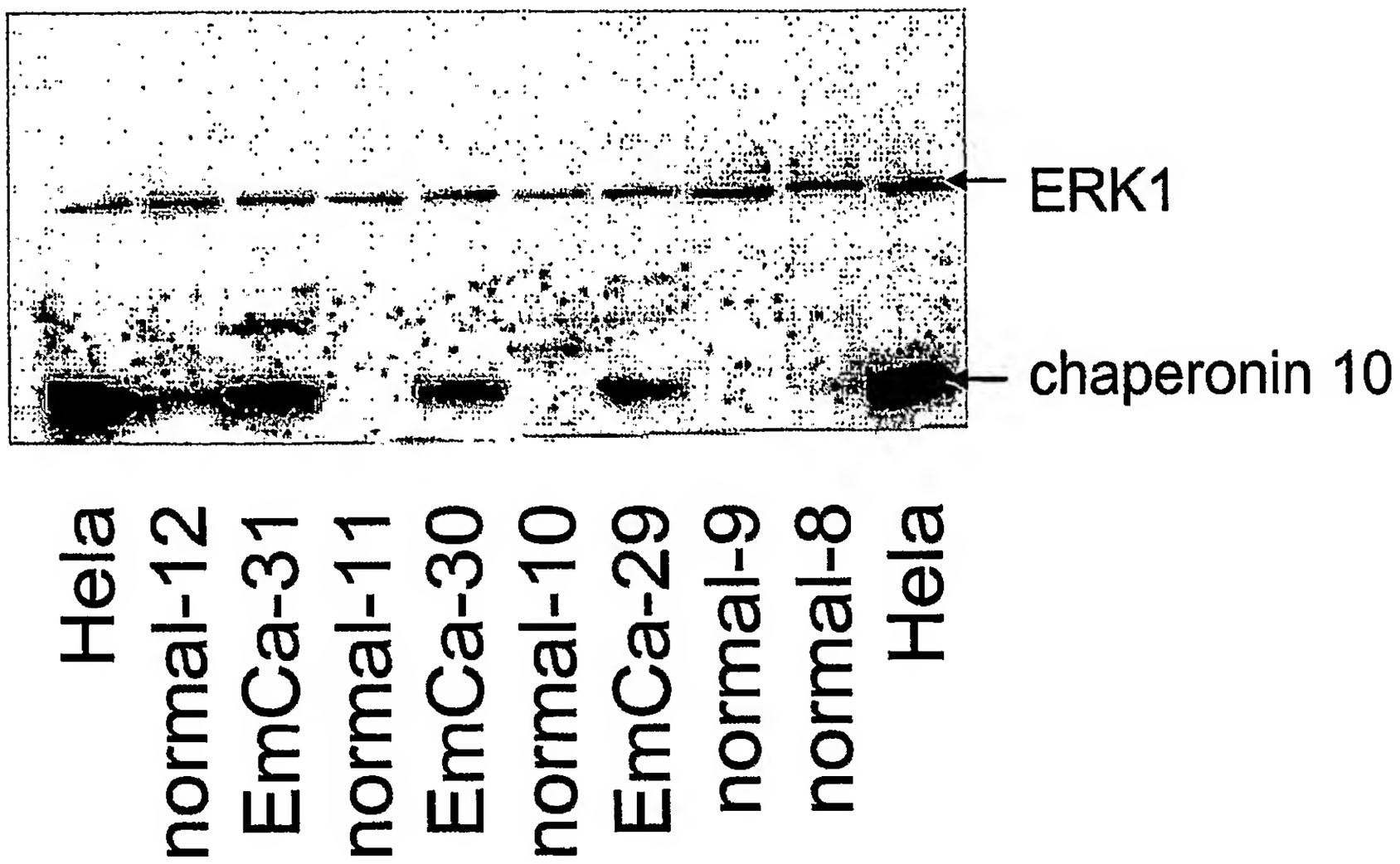

Figure 10

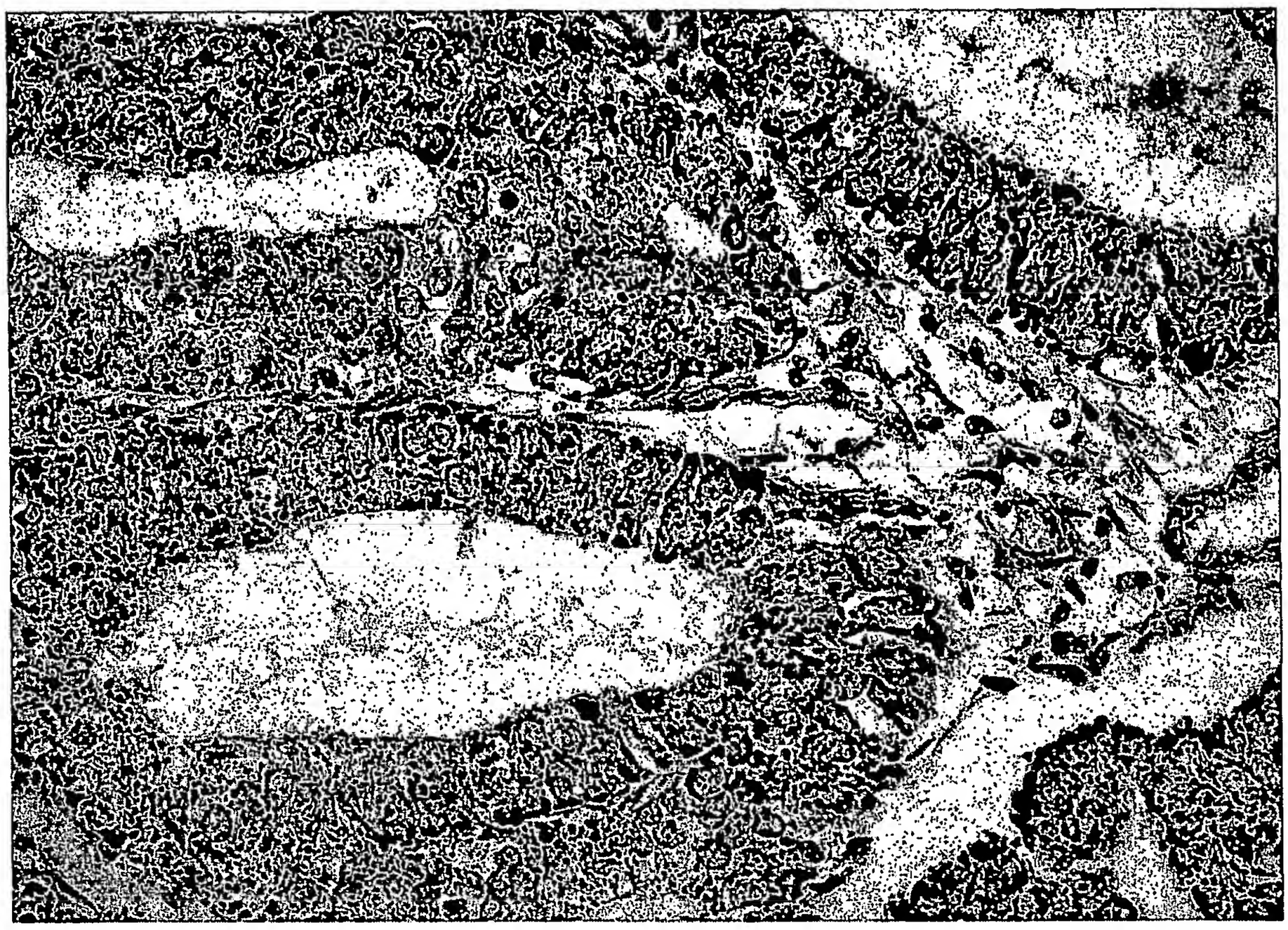

Figure 11